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SOME RESPONSES OF HONEY BEES TO ALFALFA FLOWERS

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INTRODUCTION

There is a widespread opinion that insects are creatures of instinct. However, many experiments show that some species, including the honey bee, can respond, by learning, to situations which they face in their natural environment as well as to those established by man in designed experiments. The behavior of honey bees on alfalfa flowers indicates such learning processes. The German botanist, Muller (1873), stated a theory that honey bees prefer or choose to work the flowers without tripping them. More recently, the view that honey bees learn to work alfalfa flowers was expressed by the Swedish agronomist, Åkerberg (1949), and the American journalist, Fleming (1949). Any such theory requires substantial evidence. The objectives of this study are to test the learning hypothesis, and if possible, to discover means whereby pollination of alfalfa might be improved.

Descriptions of the alfalfa flower exist (Muller, 1873, Piper et al, 1914). Basically, the flower consists of a small funnel of petals which form a corolla tube and flare outward from it. The sexual parts, including the ovary and the group of united stamens surrounding it, appear to form a stout column that is bent outward and encased under pressure between two specialized petals which form the keel. One wing petal projects on each side of the keel, and the corolla is completed by a large standard petal directly opposite the keel. The sexual parts are exposed by means of a trigger mechanism located within the corolla tube. When the trigger is pressed the keel opens, the sexual column snaps across the open corolla, and rests exposed against the standard petal. This action is irreversible. It is so sudden that early writers called it "the explosion of the flower," but we call it tripping.

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For good seed production the flowers must be tripped by insects. Burkill (1894) credits deCandolle (1832) with a theory that the alfalfa flowers trip themselves. Burkill also credits Hildebrand (1866) with recognition that the flower is especially adapted to insect pollination. However, Hildebrand's opinion was not widely accepted. A controversy on the manner of pollination ensued which remained to be resolved by Tysdal in 1940.

Nectar-gathering honey bees are relatively inefficient trippers of alfalfa flowers. Various writers have observed that they trip only 0.6 to 1.6 per cent of the flowers they visit. The author concurs with these estimates. But they contrast distinctly with values of 7 to 85 per cent obtained for certain individual bees that use simpler working methods. If nectar bees learn to avoid tripping they fail to pollinate the flowers.

Pollen-gathering honey bees are often scarce on alfalfa. Hare and Vansell (1946) showed that the pollens of some other flowers are preferred. Pollen-gathering honey bees are efficient trippers. Burkill (1894) pointed out that a bee *must* trip the flower to expose the pollen. Hence any learning involved in this case would benefit pollination. The ability of bees to gather alfalfa pollen (by learning to do so or otherwise) may be reflected in the proportion of alfalfa pollen they do gather. Thus a basis for selection and breeding of bees that are more efficient pollinators may be offered. Tests to confirm or reject the possible genetic basis of such an approach would be justified because adequate pollination of alfalfa is difficult to achieve. Higher concentrations of bee colonies are used for this purpose than for the pollination of any other crop.

METHODS AND CONDITIONS

The data presented have been selected from detailed records of 181 marked bees studied under experimental conditions in 1949 and 1950. The behavior of 736 bees was observed in duplications of the experimental condition in the field, and that of about 2000 under normal field conditions in 1951.

The experimental condition was devised primarily to confine bees to a small area where they could be repeatedly observed. Cages approximately 12 × 12 feet by 6 feet high of cheesecloth or plastic screen were erected over plots of the blooming plants. The cages were placed over the flowers several days before the bees were released. This excluded nectar-gathering and pollinating insects, so that the number of flowers in full bloom was increased, and the caged flowers built up larger volumes of nectar. (The alfalfa flower twists and withers in a few hours after it is tripped but remains fresh for four or five days if not tripped.) Colonies having a total of 4000 bees or less on 2 to 3 combs were treated to remove the older individuals which had foraged in the fields. When a bee colony was placed in a cage the success of this elimination of field bees was indicated by the absence of bees that "fought the cage" and clustered in the upper corners in obvious efforts to get out. The experimental condition differed from the normal field situation in that, as largely as possible, young bees

unconditioned by field experience were confined to a cage in which alfalfa flowers were numerous and attractive.

Bees usually began working the flowers within an hour of the time when they were liberated in the cage. Observations consisted of watching an individual bee for several flower visits to determine her habitual behavior, then marking the bee for identification and continuing to record her behavior at as frequent intervals as possible.

OBSERVATIONS OF FORAGING PROCESSES UNDERLYING THE INVESTIGATION

It is important that we distinguish nectar-gathering honey bees from pollen-gatherers so that, as their manipulations of the flowers are observed, we may judge as accurately as possible what each bee is doing or trying to do. All the worker bees are anatomically equipped to gather pollen and nectar and individual bees of the same hive engage in either activity or occasionally both at the same time. We cannot see the nectar in a bee's proventriculus, but the pollen she has gathered is readily visible as a conspicuous ball in the corbicula on each hind leg. Also a bee's intentions are indicated by the flower objectives toward which she directs her movements. The nectar-gathering bee inserts her proboscis toward the base of the corolla where the nectar is located. She then pauses a moment, apparently while the flow of nectar continues toward the honey-stomach. The bee then removes her proboscis and goes to another flower. Most of the flowers thus visited usually remain untripped, and if the flower has been previously tripped, the exposed pollen mass goes untouched. Any tiny amount of pollen visible in the pollen basket does not increase in size as the visitation of flowers continues, and pollen deposited on the bee by the tripping of flowers is cleaned off and discarded. The procedure of the pollen-gathering bee is more elaborate. She inserts her proboscis directly into the corolla of the flower, contacts the tripping mechanism, trips the flower, withdraws her proboscis, gathers the pollen by clawing the pollen mass with her forelegs, and then she moves to another flower. If tripped flowers are visited, the gathering motions may be observed, but the proboscis is not inserted. The two pollen balls grow visibly larger as the bee is followed for 20 to 40 flower visits.

Occasionally a pollen bee has been seen to make typical nectar-bee visits, that is, visiting flowers by a method which avoids tripping, or visiting tripped flowers without touching the exposed pollen mass. In these cases the bee is considered as having two objectives, nectar and pollen. Such cases were rarely seen on alfalfa flowers.

The nectar bee focuses her movements on reaching the nectar at the base of the corolla with her proboscis. There are several ways in which she may accomplish this. She may insert her tongue directly into the open corolla, she may force the mouth parts in at one side of the flower where the standard overlaps a wing petal, or she may pry in between calyx and corolla at other points. The place of insertion of the proboscis may be obscured at

any particular moment, but it is indicated by the position of the bee on the flower. The position is easily observed.

The "side position" is by far the most common. The bee perches on the flower at an angle of 20° to 40° from the line of the keel. The proboscis is inserted near one edge of the standard petal, between it and the near wing petal. The edge of the standard lies across the bee's face obscuring half of it or less (fig. 1). The throat of the flower is avoided. In a variation of the side position the bee is perched somewhat crosswise of the flower, and the tongue appears to be inserted outside the wing petal, the petal may be bent toward the keel. Or a bee may "work" the flower completely from the outside, prying her tongue in between calyx and corolla. By the use of the side position (and variations) the tripping mechanism of the flower is not contacted, and the pressure created by the bee on the flower seldom results in tripping.

The "direct" position is the commonest in the "nectar-tripper" class. The bee perches squarely on the keel and wings; she is right in line with the keel and faces the standard petal. The proboscis is inserted directly into the open corolla (fig. 2). The tripping mechanism is touched and tripping frequently occurs. The sexual column snaps toward the standard, strikes the bee on the under side of the head (Hunter, 1899) and deposits some pollen on the bee at that point (Vansell and Todd, 1946). In another nectar-tripper position the bee is inverted, that is, is upside-down if we consider the standard petal to be upright since the flower tends to gravitate so that the standard is upward. The bee perches on the standard, faces the keel, and inserts her tongue directly into the corolla. If the flower trips, the sexual column strikes the bee in the "face" and deposits a mass of pollen (fig. 3, also Vansell and Todd, 1946). The writer has observed a third nectar-tripper position in which the bee perches crosswise on the flower at right angles to the keel, and inserts her tongue directly into the corolla.

The basic difference between the side positions and the nectar-tripper positions is that in the latter the open corolla of the flower is the point of insertion of the proboscis, while in the former more difficult and devious means are used to reach the nectar. And for the nectar-tripper *the flower is a trap.* When the sexual column snaps, it not only strikes the bee but closes the space which is occupied by the bee's proboscis. The column continues to exert pressure, and the bee is held prisoner. She may free herself with a single jerk, or, in rare cases, 45 seconds of struggle may be required. The flower may be embraced and crumpled. The bee may squirm, arch her abdomen upward, stretch her neck, even "fly" vigorously to get free (fig. 4). Evans (Piper et al., 1914) and Dwyer and Allman (1933) first observed the flower as a trap. Pederson and Todd (1948) photographed a case where the struggle for freedom was apparently unsuccessful and resulted in the death of the bee. If the bee is in the inverted position (or the crosswise position) she usually frees herself with less difficulty. On this the author concurs with Åkerberg and Lesins (1949).

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 1. Bee No. 15 working an alfalfa flower from the side position. The proboscis is inserted at one edge of the standard petal which bells out to the right. The open throat of the flower is avoided.

FIGURE 2. A bee collecting nectar by the direct tripper position. The bee is in line with the flower facing the standard petal and the proboscis is inserted directly into the corolla.

FIGURE 3. The inverted nectar-tripper position. The bee is perched on the standard petal facing the keel of the flower, the proboscis is inserted directly into the corolla.

FIGURE 4. Bee C-3 struggling for release after tripping the flower from the direct position. In groping for a foothold she is bending the standard petal backward. The bee is also stretching her "neck."

In studying the behavior of bees on flowers desired data were 1) the position the bee assumed in working each flower, 2) whether or not the bee tripped the flower, 3) the type of flower visited (because some were not "trippable"), and 4) the order in which types of visits occurred so that changes in position would be indicated. To record these details a code was used as follows:

t = Flower-visit using nectar-tripper position or variations
s = Flower-visit using side-worker position or variations
T, S. = Flower visited and tripped from position t or s, respectively
p = Previously tripped flower visited
w = Withered flower visited.

A secretary was stationed outside the cage, code and notes were dictated while the observer kept the subject bee in view as continuously as possible. Bees were timed with a stop watch to furnish data on working speeds. Visits on which the details were lacking were omitted except during timing intervals. The following sections contain samples of the data obtained. The writer hopes to present more complete data in a later publication.

NECTAR-TRIPPER BEHAVIOR

A nectar-gathering bee was classified as a "side-worker" or "nectar-tripper" depending on whether she used the side positions (s) or the tripper positions (t) in a majority of her visits to flowers. Although nectar-trippers are seldom seen in the field, frequently they predominated in cages and in two of the 10 experiments made in 1950 shortly after their release *all* of the bees observed in the cage behaved as nectar-trippers.

Table 1 presents some examples of nectar-tripper behavior, selected out of 128 bees that remained nectar-trippers during observations. In the table, the code letters each representing a visit to a flower are grouped in fives for convenience. Each line records 40 consecutive observations (or less) across the page and is continuous with the line below it unless a blank space indicating a break in the sequence occurs. A break is also indicated by a horizontal space with a series of dots (.....). The bee A-2 was first observed to make 50 visits to flowers. She used the direct nectar-tripper position exclusively at this time. The bee C-4 worked even previously tripped flowers (p's) from the inverted position, and deviated from this method only once in over 200 visits during four days. However, this fixity of pattern no longer held for A-2 at the time of the second observation; an occasional inverted visit was observed. The bee was no longer definitely committed to one position. She also visited tripped flowers which were not available at the start. A-8 used the side position several times and also worked three withered flowers. Bees B-16, E-5, and G-6 used practically all the positions described.

Nectar-trippers varied widely in the proportion of flowers tripped. A-8 tripped 33 out of 49 flowers visited for a tripping efficiency of 67 per cent. A-2 tripped 8 out of 50 for 16 per cent. (In these calculations p's and w's

are excluded because they are no longer "trippable.") The tripping efficiency of individual bees ranged from 7 to 85 per cent. Coincident with this certain bees were frequently observed to avoid tripping and "beat the trap" by reaching into the corolla tubes no deeper than necessary. But the nectar reserve in the flowers was depleted in a matter of hours, and the bees then had to reach more deeply. A rise in tripping was noted for A-2 whose efficiency jumped to 50 per cent. Eleven out of 24 nectar trippers show similar rises in final visit-sequence.

TABLE 1

RECORDS OF FLOWER VISITS OF NECTAR-TRIPPERS, 1950

Bee No.	Date	Hour	Visits to flowers (See code page 262, read across)							
A-2	7-5	11:15	ttttt tttTt	ttttT tTttt	ttttt	TttTt	tttTt	ttttt	ttttT	ttttT
		p.m.	tTTpp	tTtpp	TptpT	pTttp	ppTTt	pt		
A-8	7-6	a.m.	TttTT TTTTT	ttTTt SsTSS	TtTTT TTT	tTsss	swttt	wTpTT	TTwTT	sTTTT
B-16	7-20	10:30	TTTSt	tS						
		3:30	TTTSs SttsT	stsTT STsss	Sp..... pTpTT	TSTTs TTs	sssss	ssTTT	TsTTT	Tswws
		4:00	ssssp	TTsSs	ssssT	TTTpt	ttpts	sTT		
E-5	8-23	1:00	tttTt	ttsts	TTTtt	tTTTT	tt.....	stttT	sT	
		2:25	Ttttt ttTtt ttTst tstTT	tttttT ttttts tsttt tsssst	tttTt Tpttt TTtpT wtttt	tTTtt TTTTt ss..... tttT	tTtTt TTtST sssTs	sTttt ttTTt Ttspt	TTtTt sstTs TtTtt	TtTtt tTstt tTtss
	8-24	10:30	wTtww	ttwww	wwwww	wwwww	wwwww	wwwww	w	
		1:35	wwwww	wwwww	wwwww	w				
G-6	8-30	a.m.	TTttw	TTt						
		12:00	ssssT tTtTt	sssSt ttstt	tTsss sTTtt	sTsss ttTtt	stsps tt	stttt	ssTTt	SsttT

Bees B-16 and G-6 used the side position to a considerable extent. When B-16 used the side position the flower usually failed to trip; when a nectar-tripper position was used the flower usually tripped and the bee got caught in the trap. But less than half of the t-visits of G-6 resulted in trips. These two bees are the most outstanding borderline cases in which the s-position was used to a considerable extent without becoming fixed as the predominant method of working the flowers.

The nectar-trippers were on the whole wild and nervous. This, added to their irregular appearance on the plot, increased the difficulty of recording their behavior. The bee E-5 was an erratic individual. She would touch a flower with her feet or alight on it with the apparent intention of working it,

but instead she would immediately leave, even jump away from the flower as though afraid of it. This rejection of flowers was common to nectar-trippers. The bee showed no inclination for the s-approach and had no fixed method of working flowers, but on the second day a definite preference developed for withered flowers that have no traps. The withered flower develops an abscission layer and finally drops to the ground. The stage of some of these flowers is indicated by the fact that occasionally when the bee alighted, the flower dropped and the bee and flower tumbled to the ground together. E-5 is representative of a group of nectar-trippers that developed fixations for withered and previously tripped flowers.

TABLE 2

FLOWER VISIT RECORDS OF AN UNSELECTED GROUP OF BEES IN THE ALFALFA FIELD, 1950

Bee No.	Date	Visits	Bee No.	Date	Visits (see code, p. 262)
a	8–18	62 s, 2 S	h	8–22	45 s, 2 S, 1 p, 1 t
b	"	77 s	i	"	11 s
c	"	12 s, 1 w	j	"	9 s
d	"	21 s	k	9–1	24 s, 2 p, 1 t
e	8–22	21 s	l	"	29 s, 2 w, 1 T
f	"	26 s	m	"	16 s
g	"	11 s			

It is of interest to compare the data on nectar-trippers with those of an unselected group of bees recorded in the field adjacent to the cages, table 2. The bees in the field all used the s-position. A small number (about 1 per cent) of the flowers were tripped, and t-visits were rare. The patterns of some bees were perfect during observation, but others show less perfection in the selection of flowers and in the constancy of the s-position. Occasionally nectar-trippers have been seen in the field. Tripping records of three are as follows: 30 visits—5 trips, 5 visits—4 trips, 17 visits—16 trips. Their behavior contrasts distinctly with that of the vast majority of the bees in the field.

THE CHANGE FROM NECTAR-TRIPPER TO SIDE-WORKER

Of the nectar-trippers studied in cages, 69 were recorded for only one sequence of visits, so that the chances of observing a change of approach in this group were small. Of the remaining 59 bees 23 showed a change to the side method and 3 were of doubtful classification. Nineteen other bees were side workers when first observed. Table 3 presents the patterns of some individuals that changed their approach to the flowers. Most of these changes occurred while the bee was not under observation, and the change is to be judged by the differences in pattern between sequences. B-6 (table 3) was observed three times on July 19, each time its behavior was that of a nectar-tripper. But on July 20 the s-method predominated. The initial records of some bees were fragmentary but speak for themselves.

TABLE 3

CHANGES FROM NECTAR-TRIPPER TO SIDE-WORKER AND IMPROVEMENT IN PATTERN, 1950

Bee No.	Date	Hour	Visits to flowers (See code page 262, read across)							
A-4	7-5	a.m.	tTTtT	ptTTT	TTTTt	TsssT	ssTst	tsSss	sssss	SsssS
			sSSSS	SSsss	sSSsS	sssSS	SSSsS	SSSss	sTsss	SSSss
B-6	7-19	p.m.	TTtTt	tTttt	ttttT	tsTTt	tttst	tssts	TtsTT	s
			TsTtt	tttTs	TTtts	tttT				
			Ttttt	ststt	ttTtt	tttTp	TTtTt	tTsTs	ttt	
	7-20	a.m.	wssss	ssssT	ssTtt	Tttss	sSsSs	sSsss	ssTss	T
C-11.....	8-2	a.m.	ttTsT	.....	ssss S					
		p.m.	(88 s, 4 S, 2 t, 1 T, 1 w, 1 p)							
	8-3	a.m.	(74 s, 1 S, 2 T)							
		p.m.	(77 s, 2 S)							
C-24.....	8-5	noon	ttTs							
	8-6	noon	spsss	ssssS	pssSs	sss....	.....	Tssps	sssss	s
E-7	8-23	1:20	TTsTT	ssSsS	sssss	sssTT	ssSss	sssss	sssss	sssss
			sssss	sss....	.....	ssssS	sSsss	sssss	sssst	sss
		2:50	(31 s, 2 S)							
	8-24	10:30	(38 s, 3 S)							
		p.m.	(46 s)							
E-12.....	8-23	p.m.	TTsTw	sssww	wssTt	TsssT	sssTs	sssss	ssssT	stsss
			sssss	sTsss						
	8-24	noon	sssss	sssss	pSssS	SsSss	stsss	sssss	sssps	ssSss
			sssss	tssss	sssss	s				
B-11.....	7-20	9:00	ppsss	.....	sssss	ssstT	s			
			sSsss	ttssT	tssss	sssss	sssts			
			Tstst	stsss	ststt	tsSst	sssss	sssss	ssst	
		4:45	(65 s, 1 p, 1 w, 1 T)							
	7-21 & 24		(98 s, 1 T)							

Two cases are of special interest because the change occurred while the bee was under observation. A-4 worked 15 flowers from the t-position, and tripped 11 of them. She then tried the side-position, alternated between the two methods for 10 flowers, and continued using the new method for 53 flowers with only one exception. E-7 used the t-method 4 times in the first 5 visits, twice in the next 15, and once in 170 subsequent visits. With A-4 and E-7, the new method became quickly and firmly fixed. In certain other bees the new approach developed more slowly. For B-6 t-visits resulted in repeated punishment by the trap before the bee showed any

fixation for the new method. The bees which did not show some fixation for the s-approach on or before the second day remained nectar-trippers and usually quit the flowers entirely. On the other hand any bee that used the s-approach 10 consecutive times showed a strong preference for s in all subsequent observations.

Examples evidencing improvement in pattern after initial change of approach appear in table 3. B-11 showed greater regularity in the use of the s-approach in the first afternoon and broke her pattern only once in 99 later visits. Pollen masses on the faces of a number of bees indicated they had used the inverted nectar-tripper position, but in the redords which followed immediately this approach was not used and strong fixations for s were evidenced.

When a bee changed her approach the proportion of flowers tripped dropped off quickly. The bee A-4 was the only exception to this rule. (When the flower is tripped by a bee working from the side-position the sexual column brushes past the bee, her proboscis is not caught in the corolla tube, and she suffers no apparent ill effects. S-tripping is believed to be due to the pressure of the bee's feet on the keel and wing petals.) After the initial drop accompanying the change to side position the tripping efficiency continued to approach zero, fewer flowers were worked from the tripper positions and fewer flowers were tripped from s-position. Some 1949 results illustrating the progressive drop of tripping efficiency are given in table 4. In the table, bees 1 and 7 show drops accompanying the change and improvement in the bee's technique from one day to the next. Bee 15 showed improvement in 20 minutes, and was not seen to trip a flower in 96 subsequent visits.

Side-workers and nectar-trippers were frequently observed working the same cage of flowers at the same time, although the former usually predominated except at the start of an experiment. Considerable data on the behavior of side-workers in cages exist; in general these patterns resemble those already presented. It is important that we compare the last records for individuals of table 3 with those of table 2. The bees in the field appear to have more regular patterns than most of those in cages. Their tripping efficiency was lower on the average than those of the "new" side-workers observed in cages. However, it should be noted that in final observations some of the bees in table 3 show just as regular s-patterns as the bees in the field.

It is worthy of note that many of the side-workers became visibly less nervous, more constant workers, and more easily observed as their patterns improved. The tendency to reject fresh flowers was markedly reduced. Also with the abandonment of the t-approach in favor of s, the tendency of certain bees to visit withered and tripped flowers disappeared. When the s-approach was well fixed bees showed strong preference for fresh flowers, whereas some nectar-trippers concentrated on withered flowers in the same cage at the same time.

TABLE 4

CHANGES IN TRIPPING EFFICIENCY FOLLOWING CHANGE FROM NECTAR-TRIPPER TO SIDE-WORKER, 1949.

Bee No.	Date	Time	No. flowers visited	No. flowers tripped	Tripping efficiency per cent
1	8-25	2:30	22	12	55*
		4:20	15	3	20
	8-26		86	13	15**
7	8-25	2:50	20	15	75*
	8-26	10:30	15	5	33
		3:35	21	3	14**
15	8-31	10:15	127	18	14**
		10:27	100	1	1
		1:15	66	0	0
	9-1	11:00	30	0	0

*Nectar-tripper approach predominated.
**Side-worker approach predominated.

THE WORKING SPEEDS OF NECTAR BEES IN CAGES

The working speeds of individual bees were observed by recording visits in code during periods timed by a stop-watch. Visits to all types of flowers were counted. Time samples differed in size but were seldom less than 20 visits. Figure 5 presents the changes in flower visits per minute for nectar-trippers and side-workers. The number of nectar-trippers shown in the curves is small because of the erratic behavior of this group. Numerous attempts to time them failed, the bee would either stop working or disappear. The speeds of 35 nectar-trippers ranged from 2 to 10 flower visits per minute, and over half of them worked within the limits of 5 to 7. The side-workers were more variable, the range being 6 to 23 flowers per minute for 41 bees. Half of these fell in the range of 12 to 17. Nectar-trippers made no significant increases in speed, while, with few minor exceptions, that of side-workers was striking and definite. Some bees changed so quickly to the s-approach that they were not timed before the change. But the records of A-4, C-11, E-7, F-12, and B-11 (table 3) show that improvement in s-pattern accompanied the increase in speed. The same was true of another group not shown in tables, whereas Nos. 8, H-1, H-15, H-16 that were proficient side workers when they were first timed, show relatively minor changes in speed. In bees which were timed both before and after the change to s, the change of approach came first, and the bee increased in speed afterward.

Decreases in speed by two side-workers, 16 and G-19, are shown. A shortage of flowers was noted in the case of G-19, she apparently lost time searching for flowers. There is no record for No. 16. Neither of these bees reverted to the speed of nectar-trippers.

The difference in speed between nectar-trippers and side-workers is worth examining. Frequently the nectar-tripper must consume a second or more gaining her release from the trap. But this does not explain slow-

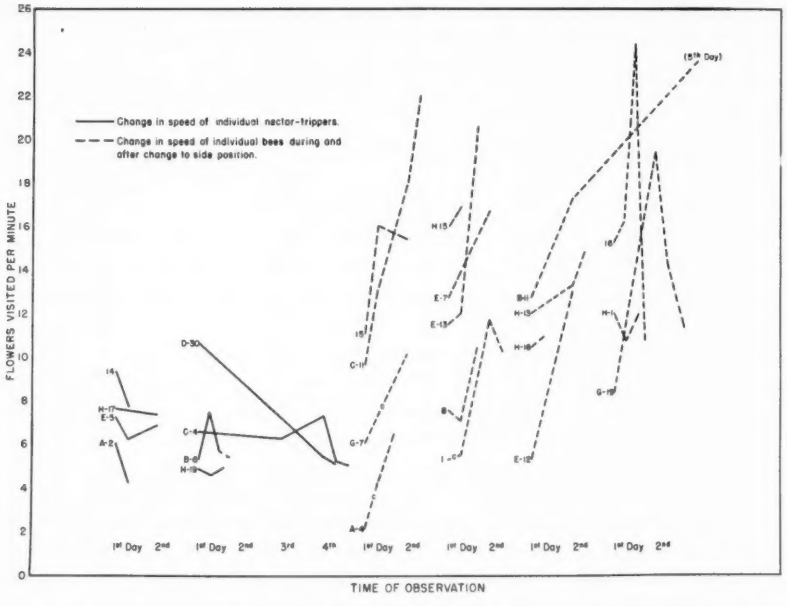

FIGURE 5. Changes in the working speeds of individual nectar-trippers and side-workers. (The letter c in some curves indicates that the change from nectar-tripper to side-worker occurred between observations. Other side-workers changed before the first timing period).

ness in those cases which could be analyzed. C-3 visited only 13 fresh flowers out of 64, the rest having been tripped; but her speed was still that of a nectar-tripper, 6.2 flowers per minute. C-4 worked from the inverted position, seldom had any difficulty with extrication, but her speed remained that of a nectar-tripper. A-4 changed to the side approach while working very slowly. Her speed increased with the change but it remained low (6.4) even though she made only one t-visit in the last 55. Hesitation, investigation of buds, slowness in selection of flowers, probing and fumbling with flowers characterize the foraging behavior of nectar-trippers.

CONFIRMATIONS IN THE FIELD

Observations were made in the field in order to test the following hypotheses:

1. That most of the nectar bees in the field are skilled side-workers.

2. That some of the bees in the hive have not yet foraged and are inexperienced nectar-trippers.

3. That the field bees communicate to the bees in the hive any new and promising sources of nectar they have discovered together with the direction and distance of the flowers containing the nectar. (These communication behaviorisms have been demonstrated by Karl von Frisch, 1950.)

If the hypotheses were correct then the sole requirement to produce unusually high tripping efficiency by nectar-seeking bees (similar to that observed in cages) should be a "new and promising source of nectar" in alfalfa flowers. It has been noted that the flowers build up large volumes of nectar when nectar-seeking insects are excluded. Hence in the caging technique we have a means of accomplishing the requirement. Upon the exposure of a previously covered plot the presumed conditions would appear to be satisfied.

Covers of cheesecloth (18 × 18 feet square) were placed in various fields, and were moved periodically (usually at 3- to 5-day intervals) to expose one plot of flowers and cover another beside it. When a plot was uncovered bees working adjacent plants quickly discovered it and abandoned the field in favor of the plot. The first bees to move in were side-workers. Some appeared to show excitement and some made an occasional t-visit but they did not revert in patterns to the methods of nectar-trippers. In a few minutes these bees would take to the air, and some would circle the plot several times before leaving in the direction of an apiary. A period of 20 to 30 minutes elapsed after exposure before nectar-trippers appeared. More bees continued to arrive, so that within two hours the population concentration on the plot exceeded that of the adjacent field. (Sometimes this would be 10–15 bees per square yard compared to 2–5 for the field.) Tripping efficiency measurements were taken, this time on plot populations. The method was to observe and record visits and trips for 10 visits or less for each of 40 bees. For comparison a like sample was taken from the adjacent field at the same time. Two observers alternated between plot and field.

The experiment was repeated 14 times in 3 fields. In 12 of these trials the per cent tripping was significantly higher on the exposed plot than in a

TABLE 5

TRIPPING EFFICIENCIES ON EXPOSED ALFALFA PLOTS AND IN ADJACENT FIELDS, 1951

Exposure No.	Date	Time	Per cent tripping efficiency	
			On Plot	In field
1	6–22	2:00	*6.93*	0.54
2	6–25	3:00	4.25	0.27
	6–27	3:00	3.03	0.97
	6–28	9:00	1.69	0.58
	6–29	3:00	1.66	0.79
3	6–29	4:10	*6.61*	(use .79 above)
4	7–25	10:00	1.95	1.07
		11:00	*5.70*	0.0
		3:00	*5.14*	1.69

Av. on day of exposure for 14 trials, 5021 visits, 4.59 per cent.
Av. tripping efficiency in fields, 18,551 visits, 1.15 per cent.

*Italics indicate significance of chi square at 1 per cent level of probability.

like sample taken from the adjacent field. Table 5 shows the results of 4 of these trials or exposures. From the table it is evident that tripping efficiencies on exposed plots were variable, and that the experiment worked better at some times and places than at others. But on the first day of exposure in a new location efficiency was always significantly higher than in the field. Efficiencies in the field also varied. In cases where the field sample was too small to show tripping at the low level of efficiency the highest field record for that exposure may be used for comparison. In exposure No. 4 the observers concentrated on getting a measure of efficiency of the first bees to enter the plot after it was uncovered. No difference was shown between plot and field. But one hour later (after nectar-trippers had arrived) tripping efficiency had risen to a significantly higher level. It stayed at that level for four hours, after which no further records were taken. Exposure 2 shows how efficiency dropped on succeeding days after exposure.

The behavior of individual bees attracted to the plots was also confirmatory. One nectar-tripper was observed to change approach from t to s, 7 of the first 10 visits seen were trips, and one out of the next 46 coincident with the change to s. Bees on plots were observed working at various speeds from 4.4 flowers per minute up to 24.0, 29 bees that tripped one or more flowers averaged 10.03 flowers per minute, 65 that tripped no flowers averaged 14.03, 60 in the field averaged 15.37. Bees showing fixations for withered and previously tripped flowers were seen, and in every case the bee showed evidence of being a nectar-tripper when she visited fresh flowers.

POLLEN-GATHERING HONEY BEES

Tripping of the flower by a nectar bee appears to be accidental, but for the pollen-gathering bee it is necessary. The tripping positions (figs. 2 and 3) are used, but tripping is not always accomplished. Frequently a pollen bee is seen to struggle momentarily, pushing on the standard petal and punching into the corolla as if in attempts to trip the flower. If tripping does not occur, she abandons the flower in favor of another. The ability of a pollen bee to accomplish her objective might be gauged by her success in tripping flowers.

Table 6 presents the tripping histories of two pollen bees. No pollen activity was seen in cages in 1950, 1X and 23 in the table are the only bees on which the record is more than fragmentary. Each of these showed higher efficiency on the second day, but no further progress. The pollen objective of 1X and 23 was evident. The duration of each bee's foraging excursions seemed to be determined by the size of her pollen load. As the ball of pollen in each corbicula reached fair size the bee would disappear or be seen to enter the hive. In 2 to 5½ minutes she would reappear in the cage with empty corbiculae. No. 1X required the pollen from 98 to 140 flowers to load her baskets. No. 23 carried home small loads at frequent intervals.

Two loads estimated at one fourth of capacity required 47 and 50 flowers, respectively. Her loads were larger the second day, two ⅔-size loads requiring 79 and 103 flowers, respectively. Signs of the nectar-gathering objective were carefully watched for but none were observed until the hive was transferred to a plot of fresh flowers of high nectar content on 23's sixth day. Side approaches and insertion of tongue into tripped flowers were then observed, 23 became a dual-purpose bee, and her tripping dropped to a new low (table 6, last line). (A pollen bee in the field was once attracted to the nectar of flowers on exposed plots. Clear evidence of dual-purpose has been observed only under these unusual conditions.)

TABLE 6

CHANGES IN TRIPPING EFFICIENCIES OF TWO POLLEN BEES, 1949

Bee No.	Time	No. of flowers visited	No. of flowers tripped	Trips per minute	No. of flowers not tripped	Per cent tripping	χ^2
1X	1st day*	247	171	5.2	76	69.3	
	2nd day	404	368	6.0	36	91.1	51.39*
	3rd day	175	157	7.0	18	89.8	0.28**
23	1st day	138	67	2.4	71	48.5	
	2nd day	293	204	3.2	89	69.7	17.90*
	3rd day	108	67	2.7	41	62.0	2.08**
	6th day	80	60	2.5	20	75.0	
		98	37	....	61	37.8	

*For number of flowers tripped and not tripped between 1st and 2nd day, 6.635 = 1 per cent level of probability for chi-square.

**For number of flowers tripped and not tripped between 2nd and 3rd day.

No. 1X reached the highest tripping efficiency of the pollen bees observed in cages. Six others ranged from 50 to 84 per cent, with an average of 69 per cent flowers tripped. No. 1X had no difficulties gaining release from the trap after tripping, but not so for 23, since hang-ups and struggles for freedom were noted even on the sixth day. The movement of an efficient pollen bee is usually a single jerk in dorsal and caudal direction. The abdomen is bent downward, not upward as that of many nectar-trippers. The bee's weight is placed directly against the pressure of the sexual column, and the pull-out is almost instantaneous. This action contrasts distinctly with the repeated struggles of the nectar-trippers.

The behavior of pollen bees in the field was recorded. Efficiencies of 13 individuals averaged 66.5 per cent of the flowers attempted. Speeds of 37 individuals ranged from 1.8 flowers tripped per minute up to 12, and averaged 5.6. Some samples indicated even lower rates than 1.8, but these were fragmentary observations of wild individuals. Indeed the nervous behavior of pollen bees as a group is similar to that of nectar-trippers and differs from that of side-workers.

GENERAL DISCUSSION

The change in habitual approach of bees to the flowers, success in avoiding the trap, followed by pattern improvement, and changes in working speed lead to the conclusion that nectar-gathering honey bees learn to work alfalfa flowers. This theory hinges on the existence of an unskilled inexperienced state in the unconditioned bee. Evidences indicate that bees use the nectar-tripper approach unless there is reason to do otherwise. The simple t-position is used by nectar bees on working the flowers of various clovers and trefoils. These flowers trip gently, the action is reversible, and there is no trap. On alfalfa flowers nectar-trippers were abundant at the start of an experiment. The fact that on occasion all bees observed were nectar-trippers has significance. The chances were against seeing this phenomenon because while one bee was being watched others were gaining experience. It is unlikely that the condition of the flowers influenced the bee's working position directly because at other times both side-workers and nectar-trippers were frequently present together. We cannot presume that a young unconditioned bee has any prior knowledge of the special mechanism of any particular type of flower. The behavior of nectar-trippers demonstrates that they do not know how to beat the traps of alfalfa flowers.

The trap provides an annoying, if not painful, experience repeated until the bee finds and uses a means of avoiding it. Some bees quickly found and used a successful means of avoidance but others failed. The experimental condition probably contributed to failure by providing some seemingly false solutions. Withered flowers contained some nectar, and after bee activity, tripped flowers were abnormally abundant. Fixations toward these types was always accompanied by evidence of failure to fix an s-pattern when fresh flowers were visited. Also with high nectar volume certain nectar-trippers found it possible to beat the trap by not reaching deeply. No bee that used the t-approach 10 consecutive times without tripping (and without experiencing the punishment) was later observed to adopt the side approach. Temporary success by the wrong method probably confused the choice of the right method.

There is further evidence on how the trap influenced the bee's choice of position. Bee G-6 (table 1, 12 M) showed some fixation for s, but 4 t's intervened. The flowers didn't trip, the error went unpunished, the experience appeared to be as satisfactory as s, and any fixation for s was broken. B-6 (table 2) enjoyed considerable success in beating the trap (t) and her progress in fixation of s was slow. On the other hand A-4 and E-7 quickly fixed good s-patterns. Each had little luck with the tripper position, A-4 escaped the trap for only ⅓ of her t's, E-7 had 6 T's to one t. Thus a bee's prior experience appeared to influence the rapidity of her change and the firmness of her fixation for the new approach. This is just as would be expected if the unhappy experience of the trap were the reason for change.

But experience appears not to be the only factor. B-16 (table 1) used s repeatedly, and her mistakes (T) were properly punished by the trap.

But she failed to fix an s-pattern. Judging by her record, her capacity to learn or retain doesn't compare well with that of A-4 or E-7.

After the initial change to the s-approach a tendency toward improvement in the regularity of pattern is evident. The flowers have ever present reminders, each t-visit is a mistake, and punishment usually follows. Thus the bees are disciplined into the new habit, and the near perfect s-patterns comparable to those of bees in the field result. But some individuals did not attain such proficiency. It is apparent that observation frequently terminated before the adaptation process in a particular bee was complete.

The data on working speeds offer a confirmation of the learning theory. It has been pointed out that the time lost struggling for release from the trap is not the major factor in the slowness of nectar-trippers. Neither is the large volume of nectar which caged flowers contain, for slow bees and fast ones were recorded under the same conditions at the same time. Nectar removal is but one of several steps a bee must take in foraging for nectar. Rapid selection of only fresh untripped flowers, perfect positioning of the bee in the same identical stance on each flower, quick insertion of the tongue without tripping, and precision in all movements characterize a bee that has acquired skill by practice. The change in speed is as would be expected in the formation of a new working habit.

The exposure of covered plots in the field duplicated the flower conditions of caging. The behavior of the side workers that discovered the plot verifies the conclusion that nectar volume is not directly associated with the working position of the bee. Also the minor influence of nectar volume on working speed was borne out. The speed of 65 non-trippers on the high-volume flowers differs only slightly from that of 60 bees of similar skill working flowers of low nectar content in the field. There was a time interval after discovery before the appearance of nectar-trippers on the plot. This was probably the time needed for the communication mechanism of Frisch (1950) to operate. The discoverers had to load nectar, mark the plot by circling, fly to the hive, and announce the discovery, together with its distance and angle to the sun. The bees in the hive had to respond to the call, and find the plot. The plot exposures supported the hypotheses on which they were based, and were confirmatory beyond the writer's expectations.

The theory that nectar bees learn to work alfalfa flowers appears to explain a number of strange phenomena. Unusual nectar-tripping behavior is sometimes noted when bee colonies are first moved into an alfalfa field, when potted plants are exposed in the apiary, and on first blooming plots of flowers. Although bees are presumed to distribute themselves according to available flowers, heavier seed set and earlier maturing have under certain conditions been noted within a radius of 40–50 yards of an apiary. (Inexperienced bees would be expected to forage close to the hive.) The theory seems to explain why nectar honey bees are relatively inefficient pollinators of alfalfa.

The pollen bees 1X and 23 both showed improvement in tripping efficiency, although 1X was the more efficient and also the faster. These data may seem small and inconclusive in view of the efficiency of some nectar-trippers. When we turn to the extrication of the proboscis the evidences are not small. Some of the pollen bees had difficulties. But why are the pollen bees *as a group* well versed in jerking free of the trap, while the nectar-trippers *as a group* experience frequent hang-ups and struggles for freedom? It seems presumptive indeed to assume that the unconditioned bees of a particular group have been imparted special knowledge of how to escape from flower traps without experience.

The maximum speed of nectar bees is about twice that of pollen bees. Within this limitation the speed range of pollen bees compares to that of nectar-trippers in cages plus that of side-workers in the field *combined*. The speed and efficiency data indicate that pollen bees do not enjoy the same measure of success that side workers do. The pollen tasks are more difficult and hazardous. The conclusion seems inescapable that their efficient rapid performance is acquired. Indeed gathering pollen from alfalfa flowers appears to be a culmination of acquired skills in which many individuals achieve dubious success.

The theory that bees learn to gather alfalfa pollen seems to explain why individual bees differ so widely. Large differences have been observed between bee colonies in the amount and proportion of alfalfa pollen gathered. To what extent the ability of the bees enters is not known. However, if differences between colonies are found to be governed by genetic factors (whether involving the intelligence of the bees or not) a basis of selection and a new approach to the alfalfa pollination problem are offered.

SUMMARY

For honey bees the alfalfa flower is a trap. When the flower trips the space in the corolla is closed, and if the space is occupied by a bee's proboscis the bee is caught and must free herself. Most nectar-gathering honey bees avoid tripping and the trap by inserting the proboscis at one side of the flower. But certain "nectar-tripper" bees used a simple method of inserting the tongue directly into the corolla where the trigger is located. Tripping frequently occurs. The subsequent struggles of the bees are described and shown in photographs.

Nectar-tripper bees were rarely seen in the field. But under conditions designed to eliminate experienced field bees the proportions of trippers observed was greatly increased, so that 128 were identified and observed in cages. About half of these furnished fragmentary data, of the other half, a third changed to the side approach. The others failed to change or were not observed after changing. The change in approach to the flowers was accompanied by a drop in tripping efficiency, improvement in habitual use of the new method and an increase in working speed. For bees that failed to change, tripping efficiency varied up or down, habitual working patterns remained variable, speed remained low, while

fixations for withered and tripped flowers appeared occasionally, and nervous behavior was general. Learning and the development of skill were indicated for those that changed. Field experiments on nectar bees offer confirmatory evidence.

The tasks of a pollen-gathering honey bee are more complicated. She must trip the flower, then extricate her proboscis from the trap before collecting the pollen. Day to day histories were acquired on only two pollen-gathering bees. Both of these showed higher tripping efficiency on the second day than the first but no further progress. Some pollen bees had difficulty with the trap but as a group they extricate their tongues with ease contrasted to the repeated struggles of the nectar-trippers for freedom. In speed, the range in flowers tripped per minute compares to the range of flowers visited per minute by side-workers plus nectar-trippers. Learning processes are indicated in the activities of alfalfa pollen bees.

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DISTRIBUTION AND BEHAVIOR OF SUPERNUMERARY CHROMOSOMES DURING MICROSPOROGENESIS IN A POPULATION OF TRILLIUM ERECTUM L.*

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INTRODUCTION

Supernumerary or B chromosomes have been described by a number of investigators in a wide variety of plant genera. Darlington and Janaki Ammal (1945) have listed one gymnosperm and more than thirty dicotyledons and fifty monocotyledons with one to twelve extra chromosomes. They refer to only one case in the genus Trillium, but additional cases have been reported (Uhl, 1939; Sparrow and Pond, 1950).

The supernumerary chromosomes discussed in this paper were found while examining meiotic stages of *Trillium erectum* for use in radiation experiments. Since such chromosomes may sometimes be confused with acentric fragments, a detailed study of the behavior of centric "fragment" chromosomes in unirradiated plants was made. It was hoped that such a study would permit an eventual comparison of radiation sensitivity of normal diploid plants with those containing one or more supernumerary chromosomes.

The normal chromosome complement in *T. erectum* has been designated A, B, C, D and E (Huskins and Smith, 1935) and, therefore, it would be confusing to refer to the small centric chromosomes in this species as B. Since their small size suggests that they might have originated by fragmentation from a larger chromosome, we arbitrarily have assigned the letter F to the supernumerary or fragment-like chromosomes described in this paper. However, it is considered probable that they are comparable to the so-called B chromosomes described by other authors for a number of plant species.

MATERIAL AND METHODS

Plants of *Trillium erectum* L. were obtained from Hump Mountain, near Elk Park, N. C., in October, 1949. The plants were stored in a cold room at a temperature of 4–6°C except for short periods while anthers were being removed. Propiono-carmine smears were made of microsporocytes or microspores from 240 plants (including 3 triploids, see p. 285) using the technique previously reported (Sparrow and Sparrow, 1949). Permanent preparations were used for most of the scoring and the majority of the photomicrographs.

*Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

RESULTS

A total of 237 diploid plants were examined cytologically at first meiotic metaphase. Of these, 193, or 81.4 per cent, were apparently normal diploids (2n = 10). The remaining 44 plants had from one to four very small supernumerary chromosomes with the following distribution: 34 (14.4 per cent) with one, 6 (2.5 per cent) with two, 2 (0.8 per cent) with three, and 2 (0.8 per cent) with four. This frequency distribution of plants with various numbers of fragments does not deviate significantly from a Poisson distribution (table 1). A short inversion was present in one of the plants containing one F chromosome (plant no. A-2617).

TABLE 1

FREQUENCY OF EXTRA (F) CHROMOSOMES IN A WILD POPULATION OF *Trillium erectum* L. FROM HUMP MOUNTAIN, N. C.

No. of F chromosomes	Observed frequency	Expected* frequency
0	193	184.031
1	34	46.560
2	6	5.890
3	2	0.497
4	2	0.031
5 and over	0	0.002

*Assuming a Poisson distribution.

In general the F chromosomes were very similar in size and were somewhat less than one-fourth the length of the smallest normal chromosome at first metaphase (figs. 1, 2, 3). In one plant the F chromosomes were larger than in any of the other plants examined (fig. 4). The centromere when visible appeared to be subterminal or median. There were no differences in the staining reactions of the normal chromosomes and the F chromosomes following the Feulgen and propiono-carmine smear methods.

The numbers of centric and acentric fragments at first and second division and microspore metaphase and the numbers of micronuclei at quartet, interphase and binucleate pollen for a number of 1, 2, 3 and 4 fragment plants are given in table 2 and the averages in table 3. As indicated in table 2 (and figs. 1–4) the number of F chromosomes is determined most readily at first metaphase. Microspore metaphase is the next easiest place to detect them (fig. 5). Careful examination of first and second anaphase will show the presence of the non-lagging F chromosomes at or near the poles. In most of the plants examined micronuclei in quartets, interphase or binucleate pollen also indicate the presence of one or more supernumeraries (see figs. 6–11).

At first anaphase most of the F chromosomes moved to the poles (fig. 6), but some lagging occurred especially in the 4-fragment plants (fig. 7). The F chromosomes at first anaphase were obviously thinner than at first meta-

TABLE 2

NUMBERS OF FRAGMENTS OR MICRONUCLEI OBSERVED OR CALCULATED PER 100 MICROSPOROCYTES FROM VARIOUS PLANTS SCORED IN 1950 AND 1951

No. of F chromosomes plant no. and year scored	No. of F chromosomes M_I	Fragments lagging at A_I		Fragments lagging at A_{II}		Quartet micronuclei		Microspore				Binucleate pollen micronuclei	
								Interphase micronuclei		Metaphase fragments			
		C^+	A^{++}	C^+	A^{++}	Small	Large	Small	Large	C^+	A^{++}	Small	Large
1 (2573) '50	100	5	3	2	1	2	3	8	0	168	8	4	0
1 (3027) '50	100	9	3	13	2	5	0	16	0	192	4	0	0
1 (2932) '50	100	3	1	25	4	25	3	36	12	180	0	0	0
1 (4082)* '51								24	0	176	8	17	4
2 (2571) '50	200	3	0			11	2	16	4				
2 (2571) '51	200	3.3	3.3			15	10	10	8				
2 (1869)* '50	200	32	10	64	2	54	9	70	6	388	12	4	0
2 (3041) '50						30	12	40	0	292	36	12	0
2 (3041) '51	200	17	3	12.5	0	12	2	16	3.7	372.9	6.8	3	6
2 (2874) '50	200	12	3	21	0	14	1	26	6				
2 (2874) '51	200	6.5	9			7.9	3.2	11.2	5.3	342.8	1.3	2	1.5
3 (2971) '50	300	10	0	86	3	75	2	40	12	469.3	0	36	0
3 (2542) '50	300	19	1			31	2	96	0				
3 (2542) '51	300	2	2.5	52	2			81.3	3	476	36	4.5	3
4 (2910) '50	397	19	2			39	6	24	4				
4 (2910) '51	303	10	4	15	8.8	11.6	4.5	14.7	6.7	772	33.6	2.5	4.5
4 (1925)* '50	400	25	1	121	1	182	1	224	0	552	16	44	4

+Centric.

++Acentric.

*These plants were obtained from the same source in 1948 (Nos. 1869 and 1925) or 1950 (No. 4082) and are not included in totals given (and table 1) for those received in 1949.

TABLE 3

AVERAGE* NUMBER OF FRAGMENTS OR MICRONUCLEI PER 100 MICROSPOROCYTES

No. of F chromosomes	No. of F chromosomes at M_I	Fragments lagging at A_I		Fragments lagging at A_{II}		Quartet micronuclei		Microspore				Binucleate pollen micronuclei	
								Interphase micronuclei		Metaphase fragments			
		C+	A++	C+	A++	Small	Large	Small	Large	C+	A++	Small	Large
1	100	5.7	2.3	13.3	2.3	10.7	2.0	21.0	3.0	179.0	5.0	5.3	1.0
2	200	15.4	5.2	32.5	0.7	24.7	6.0	32.4	4.9	354.4	11.6	4.5	1.5
3	300	10.3	0.9	69.0	2.5	53.0	2.0	64.3	6.8	472.7	18.0	20.3	1.5
4	375	19.8	2.0	68.0	4.9	103.7	3.1	121.7	2.7	662.0	24.8	23.3	4.3

*When a plant was scored at the same stage in both 1950 and 1951, the average of these results was used for each plant in obtaining the average for the stage.

+Centric

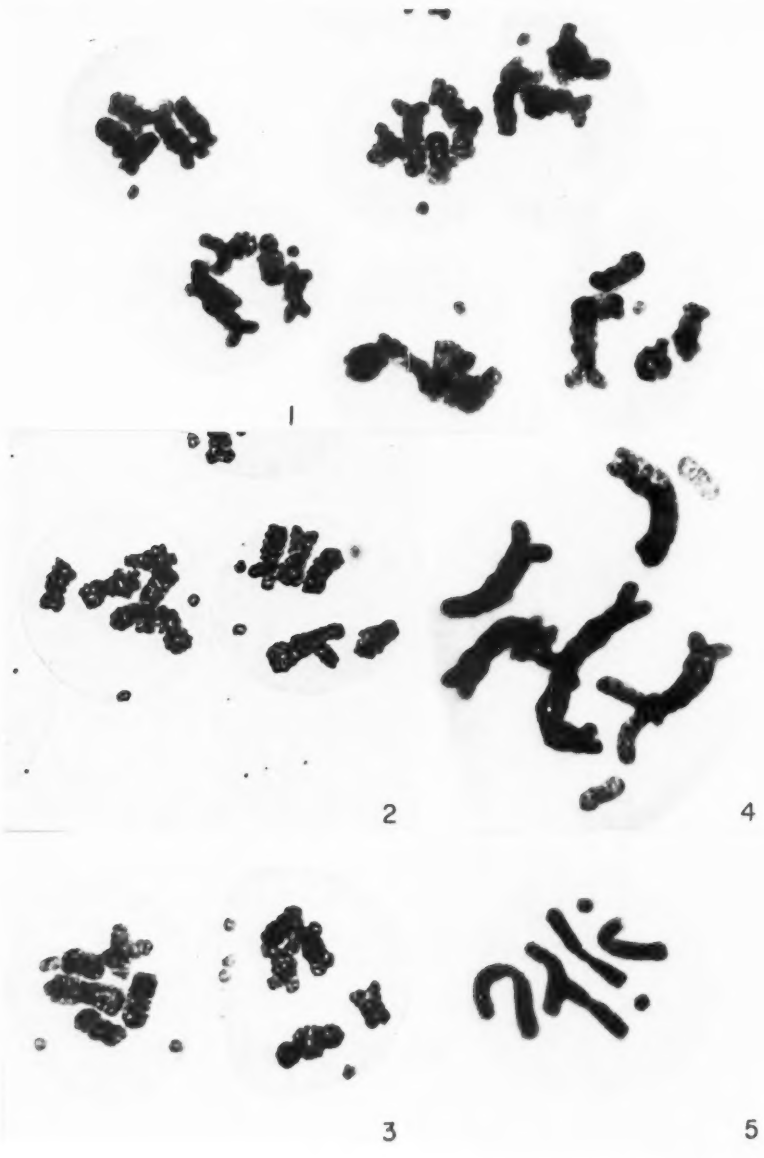
++Acentric

TABLE 4

COMPARISON OF FRAGMENT OR MICRONUCLEI COUNTS PER 100 MICROSPOROCYTES (PMC) IN PLANTS EXAMINED IN 1950 AND 1951.

No. of F chromosomes plant No. and year scored	No. of F chromosomes M_I	Fragments lagging at A_I		Fragments lagging at A_{II}		Quartet micronuclei		Microspore				Binucleate pollen micronuclei	
								Interphase micronuclei		Metaphase fragments			
		C+	A++	C+	A++	Small	Large	Small	Large	C+	A++	Small	Large
2 (2571) '50	200	3	0			11	2	16	4				
'51	200	3.3	3.3			15	10	10	8				
2 (3041) '50						30	12	40	0	292	36	12	0
'51	200	17	3	12.5	0	12	2	16	3.7	372.9	6.8	3	6
2 (2874) '50	200	12	3	21	0	14	1	26	6				
'51	200	6.5	9			7.9	3.2	11.2	5.3	342.8	1.3	2	1.5
3 (2542) '50	300	19	1			31	2	96	0				
'51	300	2	2.5	52	2			81.3	3	476	36	4.5	3
4 (2910) '50	397	19	2			39	6	24	4				
'51	303	10	4	15	8.8	11.6	4.5	14.7	6.7	772	33.6	2.5	4.5

+Centric
++Acentric


1
2
4
3
5

phase indicating that daughter chromatids had separated. No further splitting occurred at second division in contrast to the normal complement which always do. Lagging at second division was somewhat greater than at first division, but the majority of the fragments moved to the poles (figs. 8 and 9). The centric laggards formed small, deeply staining micronuclei (fig. 10), while the acentric fragments generally formed larger, more diffuse, micronuclei (fig. 11). In general, those plants with a high number of lagging centrics at second anaphase had a high number of small micronuclei at quartet (table 2). Degeneration of both types of micronuclei results in the elimination of the fragments. Since there were fewer micronuclei found in binucleate microspores, it would appear that there is less elimination of F chromosomes during microspore mitosis (table 2) than during meiosis. The behavior of the F chromosomes during pollen tube division and megasporogenesis has not been studied.

Some of the plants studied cytologically in the spring of 1950 were planted in a wooded area and examined again the following year. Comparisons of the 1950 and 1951 scorings are given in table 4. There are a number of differences between the values obtained in the two years. It is not known whether the differences are due to the small number of cells scored or to environment or to other factors.

Except for one plant there were no differences in numbers of supernumeraries observed at first meiotic metaphase. Plant number 2910 did vary at this stage. The first meiotic metaphase data obtained in 1950 indicated that it was a 4 F plant but in 1951 the analysis showed only 3 F chromosomes at this stage. However, the number of F chromosomes at microspore metaphase (772 per 100 microsporocytes) would only be expected in a 4 F plant. It is assumed that there had been some somatic elimination in the cell lineage of the anther used for first meiotic metaphase but not in the one used for microspore metaphase analysis. The possibility of somatic elimination is substantiated by the fact that a few microsporocytes still contained four fragments in 1951. These, we assume, were derived from the original 4-fragment cells. It is possible, of course, that the reverse is true, namely, that the plant was originally a 3-fragment plant and that it became chimaeric by non-disjunction of one of the F chromosomes.

The numbers of acentric fragments observed are presented in table 2. As might have been expected, there was no apparent relationship between the number of acentric fragments and the number of F chromosomes.

PLATE I

Various stages of microsporogenesis of *T. erectum* plants containing one or more F chromosomes. All photomicrographs were made from smears stained in propiono-carmine. Magnification of figs. 1–3 approximately 600 X.

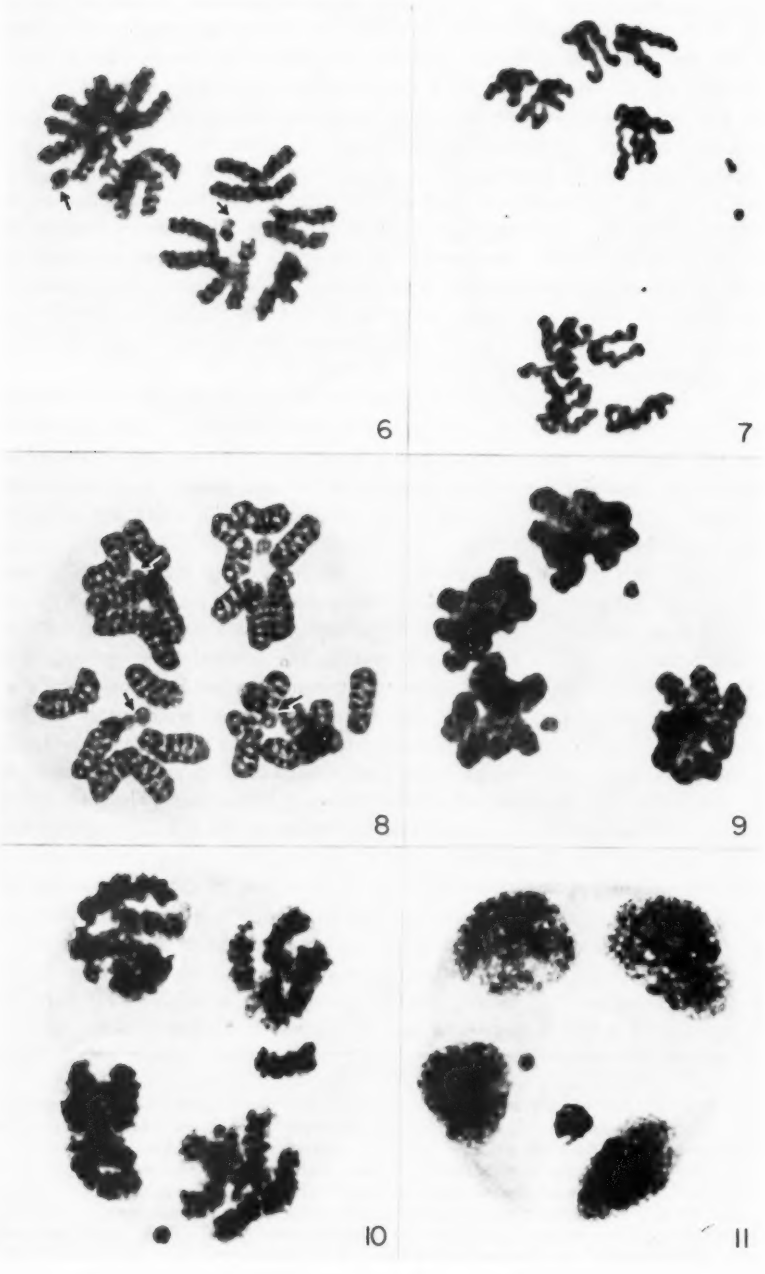
FIGURE 1. First meiotic metaphase of plant with one F chromosome.

FIGURE 2. First meiotic metaphase of plant with two F chromosomes.

FIGURE 3. First meiotic metaphase of plant with four F chromosomes.

FIGURE 4. First meiotic metaphase in plant number 3041. This plant had two extra chromosomes. A comparison of these F chromosomes with the ones shown in figures 1, 2 and 3 will show the relatively larger size found in this plant. 900 X

FIGURE 5. Microspore metaphase with two F chromosomes. 900 X


6
7
8
9
10
11

The centric fragments which persisted through second division without lagging were distributed to one or more of the four daughter nuclei and could be scored at microspore metaphase (fig. 5). If no lagging occurred during meiotic division, the number of F chromosomes at microspore metaphase should be twice the number found at first meiotic metaphase. Fig. 12 shows the relationship between the expected number and the number observed. The difference between the expected number and the observed number represents loss due to lagging during meiotic divisions. These laggards appear as micronuclei at microspore interphase. The sum of these micronuclei and of the F chromosomes observed at microspore metaphase should be equal to the expected frequency. The mean totals (per 100 microsporocytes) were 200, 386.8, 537.1 and 783.7 for the plants with one, two, three and four F chromosomes, respectively, and approach the expected values.

It was noted that great variability existed in the amount of lagging within a group of plants having the same frequency of F chromosomes. This is clearly illustrated in table 5 where the number of small micronuclei at quartet varies from 2 to 27 in 1-fragment plants and from 3 to 54 in 2-fragment plants. Similar variation also existed in the 3- and 4-fragment plants. The reason for the variability is not understood.

Supernumerary chromosomes have a tendency to pair with each other as well as to occur as univalents (table 6). They were seldom observed to pair with any of the normal complement. Trivalent associations were observed in both 3- and 4-fragment plants, but no quadrivalents were found. Plant number 2910 was exceptional in having a few cells with more or less than the expected number of F chromosomes (see discussion of this plant on p. 281).

The frequency distribution of extra chromosomes at microspore metaphase is given in table 7. Some of the microspores contain as many F chromosomes as the unreduced meiotic or premeiotic cells. A similar distribution in the megaspores would thus permit a build-up in chromosome number in a few of the offspring. Even with complete elimination during

PLATE II

FIGURE 6. First meiotic anaphase. Arrows point to the F chromosomes which have moved to the poles. Approximately 850 X.

FIGURE 7. First meiotic anaphase showing two lagging F chromosomes. Magnification approximately 850 X.

FIGURE 8. Second meiotic anaphase. Arrows point to F chromosomes at poles. Approximately 1100 X.

FIGURE 9. Second meiotic anaphase showing two lagging F chromosomes. Approximately 1100 X.

FIGURE 10. Second meiotic telophase showing an acentric fragment (at right, middle) and a micronucleus formed from a centric fragment (at bottom of picture). Approximately 1100 X.

FIGURE 11. Quartet with small dense micronucleus formed from F chromosome and much larger, more diffuse micronucleus formed from an acentric fragment. Approximately 1100 X.

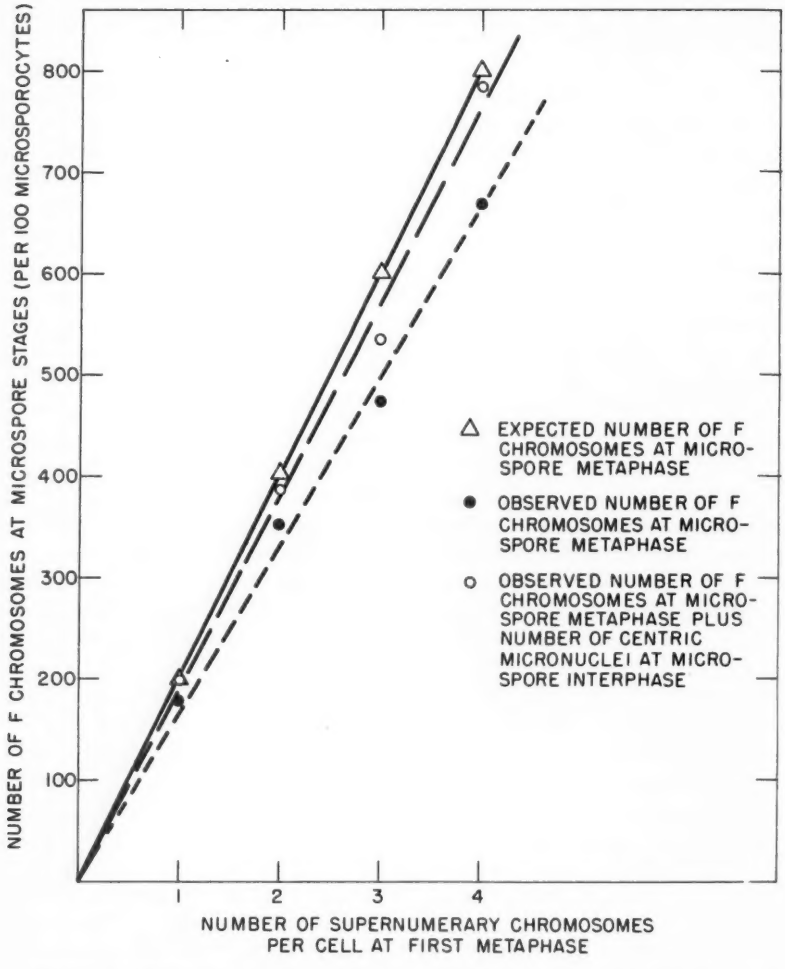


FIGURE 12. Relationship between the expected and observed number of supernumerary chromosomes in microspores (data from table III).

megasporogenesis there would be a few offspring with the chromosome number as high or higher than that of the male parent.

In order to determine if non-disjunction of F chromosomes was occurring at the microspore division the distribution at microspore anaphase was scored in 90 cells. Since only two of these showed non-disjunction of the F's it is apparent that this phenomenon is not a major factor, at least in microspore division, in building up high numbers of F chromosomes in the

TABLE 5

NUMBER OF MICRONUCLEI PER 100 PMC FOUND AT QUARTET IN 1- TO 4-FRAGMENT PLANTS

Plant	No. of extra chromosomes	Micronuclei	
		Small	Large
3113*	1	7	0
3088	1	3	0
3089	1	6	14
2523	1	12	9
2801	1	27	2
2573	1	2	3
3027	1	5	0
2932	1	26	3
3041	2	30	12
3041**	2	12	2
2874	2	14	1
2874**	2	4	4
3151	2	14	11
2676	2	3	0
2571	2	11	2
2571**	2	15	10
1869	2	54	9
2542	3	31	2
2971	3	75	2
1925	4	182	1
2910	4	39	6
2910**	4	12	5

Note: 100 Quartets scored except where noted.
*Only 97 cells scored.
**Re-examined in 1951.

next generation. Non-disjunction in somatic cells could lead to changes either above or below the "original" somatic condition but somatic cells have not been studied.

The durations of the various stages of microsporogenesis of normal and F chromosome plants have been studied. Stages up to the postmeiotic interphase show no apparent difference in timing but microspore divisions are approximately two weeks later in F chromosome plants than in the normal plants (the average length of microspore interphase in normal plants is about five weeks).

As far as could be seen, the morphology of the plants containing these extra fragments was not strikingly different from plants from the same area having the normal chromosome complement. However, further morphological comparisons with "normal" 2n plants should be made before it is concluded that no consistent differences exist.

In addition to the 237 diploid plants studied, three triploid plants were found. Two of these plants had two centric fragments and one had three. As far as the authors are aware, this is the first report of triploidy in

Trillium in North America. Triploid and tetraploid plants of other Trillium species, however, are known from Japanese collections (Gotoh, 1933; Haga, 1937). The triploidy found in *T. Hagae* (2n = 15) is thought to have arisen from natural hybridization between *T. kamtschaticum* (2n = 10) and *T. tschonoskii* (2n = 20) (Haga, 1937). However, since no tetraploids are known from North America it seems more likely that these plants arose from union of a normal haploid with a diploid gamete. Diploid and tetraploid microspores occur with a low frequency in many of the plants examined from the same collection area.

DISCUSSION

It has been suggested previously (Darlington and Upcott, 1941; Darlington and LaCour, 1941; and Darlington and Thomas, 1941) that supernumerary or B chromosomes exist in equilibrium with the normal chromosome complement in certain plant populations. The evidence in Trillium that the supernumerary chromosomes tend, on the whole, to behave in a normal fashion with relatively little lagging indicates that, as in rye (Müntzing, 1946), the centromere is fairly normal. This is not the case in maize (Darlington and Upcott, 1941), Sorghum (Darlington and Thomas, 1941) and *Poa alpina* (Hakansson, 1948b) where the defective or inadequate centromeres cause the loss of the extra chromosomes by misdivision and lagging. Even in the two Trillium plants discussed in this paper which show a great deal of lagging at quartet, the persistence of the supernumerary chromosomes through microspore division is striking. Very little lagging was seen at binucleate pollen except in three and four fragment plants. Whitaker (1936) found the number of fragment chromosomes to be constant throughout the life cycle of Tradescantia except for an occasional case of non-disjunction at meiosis.

Concerning the question of the persistence of extra chromosomes in the population, Hakansson (1945, 1948a and b), found in Anthoxanthum, *Godetia nutans* and *Poa alpina* that unpaired supernumeraries were not eliminated at meiosis as are univalent chromosomes. In Secale, however, the unpaired fragments are eliminated. A fairly large percentage of the supernumerary chromosomes in Trillium do not pair at first meiotic metaphase (table 6), and yet there is a relatively low number of micronuclei at quartet; so it would seem that in Trillium also unpaired extra chromosomes are not eliminated in appreciable numbers. Although Whitaker (1936) found no multivalent associations in Tradescantia, the fragment chromosomes failed to pair in only about 10 per cent of the microspore mother cells. In Trillium multivalent associations were observed and, in general, the F chromosomes failed to pair in a much larger proportion of the cells (table 6). Concerning the irregular pairing, White (1948) states that it is usually characteristic of supernumeraries and attributes this behavior to the absence of a "frontier" which is necessary for crossing over. This may be the case in *Trillium erectum* also since chiasmata were not observed in the paired supernumeraries.

TABLE 6

PAIRING OF F CHROMOSOMES AT FIRST MEIOTIC METAPHASE IN 2-, 3- AND 4-FRAGMENT PLANTS.

Chromosome associations	Number of F chromosomes						
	2		3		4		
	Plant No. 2571 (1950)	Plant No. 3041 (1951)	Plant No. 2542 (1950)	Plant No. 2542 (1951)	Plant No. 1925 (1950)	Plant No. 2910 (1950)	Plant No. 2910 (1951)
I						1	
I + I	73	79				1	1
II	27	21					
I + I + I			28	49			17
II + I			57	45			78
III			15	6		1	
I + I + I + I					42	45	1
II + II					8	2	
III + I					10	0	0
II + I + I					40	48	3
IV					0	0	
I + I + I + I + I						1	
I + I + I + I + I + I						1	
Total cells	100	100	100	100	100	100	100

Many workers have found that a high percentage of extra chromosomes maintain themselves in the population by entering the generative nucleus at the microspore division. Müntzing (1945), working on rye, found directed non-disjunction on both the male and female sides, while Randolph (1941) found that in maize it occurs only on the female side and in Anthoxanthum, Ostergren (1947) found it only on the male side. Very little evidence of non-disjunction of the supernumerary chromosomes was observed in Trillium.

It is interesting that in the case of *T. erectum* a high frequency of supernumerary chromosomes occurs in these plants from Hump Mountain, N. C., while none are known to occur in plants of this species collected from northern regions, e.g. Vermont, New Hampshire and Quebec (Sparrow, unpublished and personal communications from Huskins and Boothroyd).

In general there is very little beneficial genetic effect to be found in plants with extra B chromosomes. Such chromosomes are generally believed to be genetically inert or sub-inert (Ostergren, 1947; Hakansson, 1948a and b; Müntzing, 1943; Randolph, 1941). In rye (Hakansson, 1948), Anthoxanthum (Ostergren, 1947) and maize (Randolph, 1941) fertility and vigor are reduced. Longley (1927) states that in *Zea mays* pollen viability does not appear to be associated with chromosome number. Müntzing (1943) finds that only when a high number of extra chromosomes are present can even a slight effect on vegetative development be seen; however, he did find a negative correlation between the number of B chromosomes and the kernel weight per plant, the number of kernels per ear, the per cent of seed setting and pollen fertility. In maize, Randolph (1941) found that the B

chromosomes were not necessary for growth and reproduction, and he reported an increase in germless and defective seeds with an increase in the number of B chromosomes.

As has been stated above no morphological effect was noted in these F chromosome Trilliums, but no examination of the effect on fertility was made. A delay in later stages of microsporogenesis in F chromosome plants was observed. The cause of this delay is not understood. A similar delay in division rate has been found in B chromosome plants of Sorghum (Darlington and Thomas, 1941), rye (Müntzing, 1946) and Anthoxanthum (Ostergren, 1947).

TABLE 7

FREQUENCY DISTRIBUTION OF F CHROMOSOMES AT MICROSPORE METAPHASE IN *Trillium erectum*

No. of F chromosomes per PMC	Plant No.	No. of cells scored	Frequency distribution of F chromosomes†					
			0	1	2	3	4	5 or more
1	2573	100	58.0	42.0	0	0	0	0
1	3027	100	54.0	44.0	2.0	0	0	0
1	2932	100	55.0	45.0	0	0	0	0
1	4082*	100	58.0	40.0	2.0	0	0	0
Average			56.25	42.75	1.0	0	0	0
2	1869	100	18.0	67.0	15.0	0	0	0
2	3041	100	35.0	57.0	8.0	0	0	0
2	3041**	177	23.2	60.5	16.4	0	0	0
2	2874**	335	16.4	81.8	1.8	0	0	0
Average			23.15	66.58	10.3	0	0	0
3	2971	75	18.7	46.7	32.0	2.7	0	0
3	2542**	200	15.0	55.0	26.0	4.0	0	0
Average			16.85	50.85	29.0	3.35	0	0
4	1925	100	14.0	43.0	34.0	9.0	0	0
4	2910**	143	1.4	25.9	53.1	17.5	2.1	0
Average			7.7	34.45	43.6	13.25	1.05	0

Note: When more or less than 100 cells were scored per plant percentages are given.

*First examined in 1951.

**Re-examined in 1951.

†i.e. actual distribution observed at microspore metaphase.

Little is known of the origin of these extra chromosomes, but it has been suggested by McClintock (1933) that the association of non-homologous parts in triploids, trisomics and monosomics may give rise to translocations which in turn may result in supernumerary chromosomes. In Tradescantia, Whitaker (1936) observed microspores containing six normal univalents, one ordinary fragment and the two arms of a duplicate chromosome. One arm, having the fiber attachment, is oriented on the metaphase plate. The other arm will be lost and the surviving chromosome complement will include the new long fragment with the fiber attachment. The loss of the distal arm of this new fragment would produce a fragment chromosome. It

is possible that the F chromosomes may arise in Trillium also through loss of distal portions of normal chromosomes by spontaneous fragmentation, preceded or followed by abnormal segregation. A low frequency of spontaneous fragmentation is known to occur in this species (Sparrow and Sparrow, 1950).

The presence of supernumerary chromosomes in a group of X-rayed plants presents certain problems. Little error is introduced in the analysis of the effect of the irradiation when scoring is done at first meiotic anaphase or binucleate pollen, where little lagging of centric fragments occurs. If, however, scoring is done at microspore metaphase where a high percentage of the supernumeraries are readily visible, it sometimes becomes difficult to distinguish between the radiation-induced acentric fragments and centric F chromosomes. For critical experiments we have adopted a screening test to be sure that the material being irradiated does not contain any F chromosomes. This is a time consuming but necessary precaution.

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SUMMARY

Two hundred and thirty-seven *Trillium erectum* L. from Hump Mountain, N. C. were examined cytologically at first metaphase. Of these, 193 had the expected five pairs of chromosomes. Forty-four (18.6 per cent) plants contained from one to four small supernumerary chromosomes as follows: 34 (14.4 per cent) with one, 6 (2.5 per cent) with two, 2 (0.8 per cent) with three, and 2 (0.8 per cent) with four. In addition, three triploids were found, two of which had two supernumeraries and one had three.

A detailed study of the behavior of the supernumeraries has shown them to have the following characteristics:

1) They are mostly similar in size.
2) They stain with carmine and Feulgen in a manner similar to the chromosomes of the normal complement.
3) The centromere appears to be subterminal or median.
4) They show a tendency to pair with each other but generally not with the normal chromosomes.
5) A low percentage is eliminated during microsporogenesis, but little elimination occurs during microspore mitosis.
6) Their behavior from plant to plant is quite variable with respect to lagging and subsequent loss.

It is pointed out that collections of this same species from northeastern United States and certain adjacent areas in Canada have not yet shown the presence of extra chromosomes. It thus appears that the extra chromosomes are more localized geographically than the species as a whole but the limits of their distribution are unknown.

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THE PROBLEM OF ENVIRONMENT AND SELECTION

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The choice of the environmental conditions under which to practice selective breeding presents a problem of practical importance in many branches of livestock improvement, but for which genetical theory has not yet provided a satisfactory solution. The problem is whether the best results will be achieved when selection is carried out under the conditions in which the improved breed will eventually be required to live; or whether better results may be attained under some other conditions, for example under conditions more favorable for the expression of the desired character. On the one hand, one may argue with Hammond (1947) that an environment favorable to the expression of the desired character will allow more rapid progress under selection; and that if the improved breed is then transferred to less favorable conditions it will have attained a higher level of performance than could have been attained by the same amount of selection under the less favorable conditions. On the other hand, most geneticists would probably argue that performance (in respect of milk production, growth rate or any other character) in a favorable environment has a different genetic basis from performance in an unfavorable environment: a superior genotype in one environment could not be expected to be superior in a different environment. The genetic situation would thus be regarded as a case of genotype-environment interaction; and if the environments differed much, as for example temperate and tropical climates, or high and low planes of nutrition, an interaction large enough to vitiate Hammond's argument would be expected. It would therefore generally be recommended that selection should be carried out under the environmental conditions in which the improved breed is destined to live.

The possible existence of genotype-environment interactions has been widely recognized and often discussed: Haldane (1946) has specified a number of different forms which the interaction may take, and has discussed their bearing on livestock breeding; and Lerner (1950) has given a general discussion of the problem in relation to poultry breeding. But the concept of genotype-environment interaction does not seem to lead easily to a solution of problems connected with selection; differences of heritability cannot be taken into account, and a precise evaluation of Hammond's argument in genetic terms cannot be arrived at.

It is the purpose of the present paper to point out that, if only two different environments are considered, the interaction may be expressed as a genetic correlation. When so formulated the genetic aspect of the situation becomes clear, and a quantitative evaluation of the efficacy of different methods of selection may be easily obtained by the procedures already

devised for dealing with genetic correlations. A similar treatment of genotype-environment interactions was derived independently by Reeve and Robertson, working on body size in Drosophila reared at different temperatures, and using progeny tests to estimate the importance of genotype-environment interactions (Reeve, in press). A more extended mathematical treatment of the relation between the analysis of variance and correlation techniques is given by Reeve and Robertson (in press).

Suppose that a character, for example growth rate, is measured in two different environments, for example high and low planes of nutrition. The two measurements are then not to be regarded as representing a single character, but two different characters; and the two environments are to be regarded as "treatments" which have to be applied in order that the characters may be measured. The solution of the problem now follows the method of analysis widely used in dealing with the behavior of correlated characters under selection (see for example Hazel, 1943, and Dempster and Lerner, 1947.

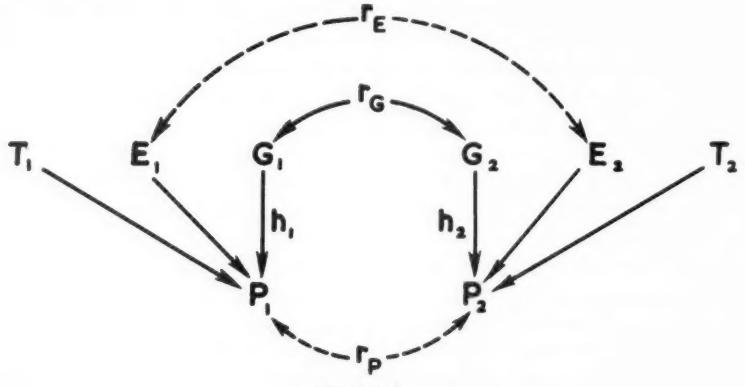

FIGURE 1.

The situation may be formally represented in a diagram (fig. 1), in which P_1 and P_2 represent the phenotypes of the two characters as measured in the two environments. There are three sources of variation affecting each of the two phenotypes: the two genotypes, G_1 and G_2; environmental differences within each treatment, T_1 and T_2; and environmental differences not associated with the treatments, E_1 and E_2. The two phenotypes are correlated through a genetic and, in theory at least, through an environmental path. The two genotypes, G_1 and G_2, are connected by a correlation, r_G. This genetic correlation, together with the path coefficients, h_1 and h_2 relating genotype to phenotype (which are the square roots of the two heritabilities) are all that is needed for the solution of the problem. But the completion of the diagram, showing all the sources of variation influencing the two phenotypes, makes for greater clarity. Variations within one treatment are not correlated with variations within the other,

so no correlation path connects T_1 with T_2. But environmental differences not associated with the treatment are correlated, and E_1 is connected with E_2 by a correlation, r_E. To illustrate this point one may think of the treatment as being the plane of nutrition on which the animals are reared from weaning to maturity. Then the environmental correlation will operate through such factors as temperature and pre-weaning maternal influences.

In situations of the sort under discussion the two phenotypes will not be measurable in one and the same individual, and the environmental and phenotypic correlations will therefore have no reality; but we may regard them as being at least potentially existent in every individual. The genetic correlation is, of course, actually present in every individual since all individuals possess genes corresponding with both genotypes, even though only one genotype can find expression in a phenotype in any one individual. The complete path diagram is now exactly like one illustrating a simple case of correlated characters, except that the environmental source of variation has been split into two, one part associated with the treatment and the other not, and that the environmental and phenotypic correlations have no real existence. It is necessary to make the assumptions discussed by Lerner (1950, p. 83), that environmental variations, whether associated with the treatment or not, are uncorrelated with variations of genotype; and that the environmental variations within each treatment are small in comparison with the difference between the treatments, for if this were not so the interaction between genotype and environment which operates between the treatments would become important also within the treatments. In other words, it is assumed that the complete determination of the phenotype is specified by the formula $\sigma_P^2 = \sigma_G^2 + \sigma_E^2 + \sigma_T^2$.

Now suppose a population to be divided into two strains, and selection to be made for the phenotype P_1 in one strain and for P_2 in the other, the technique and intensity of selection being the same in the two strains. Then after some generations of selection, some animals of each strain are subjected to the treatment appropriate to the other strain, so that both phenotypes are measured in both strains. Suppose, further, that we are interested in the character P_1, which may be thought of as growth rate on a low plane of nutrition. The problem is, then, to find the amount of improvement in P_1 obtained as a correlated response to selection for P_2, and to compare this with the improvement of P_1 obtained as a direct response to selection for P_1 itself. By an application of the rules of path coefficients, the magnitude of the direct response may be written

$$\Delta G_1 = i h_1 \sigma_{G_1} \tag{1}$$

(see Hazel, 1934); and the magnitude of the correlated response may be written

$$\Delta' G_1 = i h_2 r_G \sigma_{G_1} \tag{2}$$

(This formula may also be readily derived from the formula given by Lerner (1950, p. 236) by rearrangement and substitution for the direct response,

ΔG_2, as in (1) above.) The value of $\bar{i}$, the selection differential in standard measure, will be the same in the two cases since the manner of selection is assumed to be identical in the two strains. The ratio of the correlated to the direct response therefore becomes

$$(3) \qquad \frac{\text{correlated response}}{\text{direct response}} = \frac{\Delta' G_1}{\Delta G_1} = \frac{h_2}{h_1} r_G$$

Thus we can evaluate the correlated response relative to the direct response simply in terms of the two heritabilities and the genetic correlation; and we can see clearly what conditions must be fulfilled if the correlated response is to be greater than the direct response. Some special terms are needed for clarity. Let the "primary environment" be the conditions under which the improved breed is destined to live, e.g. low plane of nutrition. The "desired character" is then the phenotype, e.g. growth-rate, measured in the primary environment. Let the "secondary environment" designate the conditions under which selection is made, but under which the improved breed is not required subsequently to live, e.g. high plane of nutrition. The "direct response" is then the improvement in the desired character attained by selection in the primary environment, and the "correlated response" is the improvement in the desired character attained by selection in the secondary environment. The quantitative solution of the problem then leads to the following conclusions.

(1) An advantage of selection in the secondary environment would accrue only through an increase of heritability; but the increase of heritability would have to be great enough to offset the loss of efficiency through selection being made for a character that has not exactly the same genetic basis as the desired character. In fact the product, $h_2 r_G$, must be greater than h_1 where h is the square root of the heritability, and 2 refers to the secondary environment. (2) No general statement about the relative merits of the two methods of selection can be made: each case must be treated individually. The full solution requires the estimation of three genetic parameters, the two heritabilities and the genetic correlation. The determination of the genetic correlation could be made by methods similar to those used by Hazel (1943) and Dempster and Lerner (1947), but it might present practical difficulties. (3) Though each case requires its own solution, it seems probable that there will be few cases when direct selection will not yield the better result. If the two environments are very dissimilar, e.g. temperate and tropical climates, the genetic correlation will be low, and the difference in heritability would have to be very great for the correlated response to be greater than the direct. The expectation of a much higher heritability in the secondary environment would be the only justification for favoring selection in the secondary environment.

The common situation in which the experimenter or breeder seeks to improve heritability by the control of the environmental variation will be seen to be a special case of the general problem. If the nature of the environment is not altered, but merely its variability reduced, the genetic correla-

tion will be unity, and the rate of progress becomes proportional to the square root of the heritability, as is already well known. The insertion of the genetic correlation into the formula enables one to allow for any effect that the control of the environment might have on the genetic basis of the character measured.

An experiment with mice has recently been described (Falconer and Latyszewski, in press) in which two strains were selected for body-weight, one on a high plane of nutrition and the other on a low. After some generations of selection the performance of each strain on the other diet was measured. It was found that neither of the two correlated responses was as great as the direct response; but when compared with the corresponding direct response, the correlated response following selection on the low plane was much greater than the correlated response following selection on the high plane. It was found, further, that heritability was higher on the low plane than on the high. The causal connection between these last two findings was not realized when the experiment was described, but it becomes quite clear when the problem is treated as one of genetic correlation and correlated responses. Neither the heritability nor the responses were very exactly determined, but they may serve for illustrating the method of treatment.

The observed heritabilities were 0.2 on the high plane and 0.3 on the low, and the ratio of the correlated to the direct response for weight on high plane was about 0.8. Therefore, substituting in equation (3), where 2 refers to low plane and 1 to high plane,

$$0.8 = \frac{\sqrt{0.3}}{\sqrt{0.2}}\, r_G,$$

whence $r_G = 0.65$.

The other correlated response, that is the change of weight on low plane following selection on high plane, was thought to be zero. This can now, however, be seen to be impossible. Taking the value found for r_G and substituting again in equation (3), where 2 now refers to high plane and 1 to low, we find the expected ratio of the correlated to the direct response of weight on low plane to be about 0.5. This correlated response was very inexactly determined, and the observations were actually not inconsistent with this expected value.

An experiment designed to provide reliable estimates of the two heritabilities and of the two direct and correlated responses, would yield two separate estimates of the genetic correlation, and would therefore provide a check on the validity of the treatment of the problem outlined in this paper. Such an experiment is now being made with mice. The first stages of a similar experiment with pigs, in which selection for growth rate is being made on high and low planes of nutrition, are reported by Brugman (1950). The outcome of this experiment will be awaited with interest.

SUMMARY

Situations involving an interaction between genotype and environment may be treated by the methods of genetic correlation, if only two different environments are considered. Formulation of the genotype-environment interaction in terms of a genetic correlation leads easily to a solution of problems connected with selection. In this way a precise answer can be given to the question whether it is better to carry out selection in the environment in which the improved breed is required eventually to live, or in some other environment more favorable to the expression of the desired character. Performance in the two environments is regarded as two different characters which are genetically correlated. Selection for one character will then bring about a correlated response of the other character. The magnitude of this correlated response may then be compared with that of the direct response to selection for the desired character itself. The ratio of the correlated to the direct response may be expressed in a simple formula involving the square roots of the two heritabilities and the genetic correlation. It is possible for the correlated response to be greater than the direct response, but it seems probable that this will seldom happen. The expectation of a great increase of heritability would be the only justification for favoring selection in an environment other than the one in which the improved breed is required to live.

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THE INTRINSIC RATE OF NATURAL INCREASE FOR THE HUMAN LOUSE, PEDICULUS HUMANUS L.

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In 1940 the late A. J. Lotka called attention to the advantages of using the intrinsic rate of natural increase, or the rate of increase per head in a population which has attained a stable age distribution, as a measure of population growth (Lotka, 1943). But curiously enough there is no reference to this important parameter in the most recent survey of progress in the field of population ecology (Hutchinson and Deevey, 1949), and only a few papers dealing with this concept have been published to date. This is no doubt due in part to the fact that calculation of the intrinsic rate of natural increase requires a knowledge of age-specific mortality and fecundity rates, and data of this sort are not common. In fact, the list of animals for which life-table information of any kind is available is an extremely short one; to those mentioned by Hutchinson and Deevey may be added the rice weevil *Calandra oryzae* (Birch, 1948) and the flour beetle *Tribolium castaneum* (Leslie and Park, 1949). In the majority of these cases, life-table data have been given for a portion of the life span only. Complete records are relatively rare, even for laboratory-reared species. To the best of our knowledge, such information has not yet been published for a parasitic organism. For these reasons, it seems desirable to make available the material presented in this paper.

Several years ago, one of the authors (F. C. E.) had the opportunity of rearing in the laboratory a considerable number of human lice, *Pediculus humanus* L. Satisfactory information about all stages of the life history was obtained in sufficient quantity to provide a complete life-table for this species as it existed under controlled conditions. The strain of louse used for this study was acquired through the kindness of Messrs. H. H. Stage and G. H. Culpepper of the Bureau of Entomology, United States Department of Agriculture. The majority of the life-table information was secured in the course of an experimental program directed by Dr. Paul Gyorgy, Department of Pediatrics, University of Pennsylvania School of Medicine, and supported by a very generous grant from the Josiah Macy, Jr. Foundation of New York. Grateful acknowledgement is made to these persons, as well as to the many members of the staff and institution of Clinton State Farms, New Jersey, who cooperated in the program. Further information on certain life history aspects was obtained from subsequent observations carried on at Haverford College, Haverford, Pennsylvania, with the assistance of Robert M. Davenport.

METHODS AND DATA

The general features of the biology of *Pediculus humanus* are well-known and have been summarized by Buxton (1939). The life cycle consists of

the egg, three larval instars, and the adult stage. The species is an obligate parasite of man, but Culpepper (1946a) has successfully reared through many generations a strain that fed on domestic rabbits. The human louse can readily be maintained in the laboratory, large numbers can be housed in a very small space, and the rapidity with which it completes its life history makes it potentially useful as an experimental animal.

The rearing technique employed in securing the present data was patterned closely after that used by Culpepper (1944). The lice were maintained in a constant temperature cabinet at a temperature which rarely went above 30.5° C. or below 29.5° C. and at a relative humidity which varied between 30 and 55 per cent but which generally approached the higher value. The conditions closely approximate those at which Culpepper (1946a) obtained maximum average fecundity and longevity and which may be considered optimal for the species. The lice were kept in uncrowded colonies on small (1.5 × 1.5 inches) patches of woolen cloth in glass beakers, which were removed from the cabinet for about 15 or 20 minutes in the early morning and for a similar period in the late afternoon to allow the insects to feed on the arms or legs of volunteer hosts. Counts of surviving individuals in each colony were made daily, and dead lice were removed as well as all eggs produced during the preceding 24-hour period. Each day's egg production was kept separately and formed the basis of a subcolony of which a number were kept for observations on larval development.

The data pertaining to duration of the several life history stages have been summarized in terms of mean values. In all cases where it has seemed useful to indicate the precision of the mean estimate, the standard error ($\sigma_{\bar{x}}$) has been used. The standard deviation (σ_x) is also given, to show the extent of variation in the population.

Adults. The records of adult survival used to prepare the accompanying life-table were obtained from 800 freshly emerged adult lice reared in 1947 when the temperature cabinet was being maintained at the Clinton State Farms. These lice consisted of equal numbers of each sex and were distributed in eight colonies of 50 males and 50 females each. The basic survival data are shown in table 1. Since the colonies were not counted more often than once a day, it is assumed that, when first observed, mature lice had already had on the average 0.5 days of adult life. The maximum duration of adulthood in these colonies was 43-44 days for a male and 45-46 days for a female. The mean duration of adult life in days was 17.645 ± 0.398 for males and 17.575 ± 0.459 for females, with respective standard deviations of 8.0 and 9.2 days. The difference between these two means is not statistically significant.

Eggs. The 400 females in the eight colonies referred to above produced a total of 32,595 eggs, or an average of 81.5 eggs per female. The total daily production was observed for each colony, and the mean number of eggs per female per day, recorded for the eight colonies as a whole, is also shown in table 1. Lice of this species do not normally lay eggs on their first day of adult life. Egg production rose steadily from less than 2 per

TABLE 1

ADULT SURVIVORSHIP AND FECUNDITY IN *Pediculus humanus*

Mid-point of age class, in days after emergence from larva	Number alive at mid-point of age class		Eggs per female per day	Mid-point of age class, in days after emergence from larva	Number alive at mid-point of age class		Eggs per female per day
	♂♂	♀♀			♂♂	♀♀	
0.5	400	400	0.0000	24.5	86	94	4.6064
1.5	398	396	1.8686	25.5	74	85	4.3529
2.5	398	395	3.2557	26.5	65	72	4.4167
3.5	392	385	4.4324	27.5	55	66	4.7424
4.5	386	379	4.7256	28.5	42	59	4.7600
5.5	370	370	5.0541	29.5	33	53	5.4151
6.5	366	358	5.4665	30.5	22	47	4.7872
7.5	357	351	5.2336	31.5	18	38	4.8684
8.5	350	338	5.3757	32.5	11	27	5.4444
9.5	343	322	5.6491	33.5	5	19	5.7895
10.5	322	306	5.3725	34.5	4	17	3.6471
11.5	298	292	5.7534	35.5	3	11	5.2727
12.5	287	270	4.7778	36.5	1	9	10.1111
13.5	277	248	5.2984	37.5	1	8	8.0000
14.5	253	232	5.3405	38.5	1	8	3.3750
15.5	234	210	5.6333	39.5	1	6	5.6667
16.5	216	188	5.8457	40.5	1	5	4.4000
17.5	193	173	6.0578	41.5	1	5	4.4000
18.5	168	158	5.4747	42.5	1	5	3.0000
19.5	154	141	5.8794	43.5	1	4	4.2500
20.5	138	138	5.4420	44.5	0	3	4.3333
21.5	131	124	5.6694	45.5		1	4.0000
22.5	114	114	5.0789	46.5		0	0.0000
23.5	98	100	5.2800				

female on the second day to more than 5 on the fifth day, maintained this level throughout most of the reproductive period, and dropped off slightly toward the close.

A total of 28,197 eggs were observed throughout the egg period and 24,730 of these, or 87.7 per cent, hatched. The duration of the egg stage was determined for 24,645 eggs, as shown in table 2. Eggs hatched in from 5 to 12 days. The mean duration in days for the egg period was 8.015 ± 0.009, with a standard deviation of 1.4 days.

Larvae. Four of the eight colonies provided information on larval mortality. Of 804 larvae observed throughout the larval period, 694 or 86.3 per cent matured and emerged as adults. It was not possible to keep records of individual larvae, and the duration of each of the three larval instars was not determined. In 1948, however, after the temperature cabinet had been transferred to Haverford, the interval between hatching from the egg and each of the three molts was observed for a smaller number of larvae, as shown in table 3. The mean interval in days between hatching and molting was 5.230 ± 0.038 for the first molt, 8.627 ± 0.031 for the second molt, and 12.812 ± 0.036 for the third molt. From the figures the length of each instar

TABLE 2

DURATION OF THE EGG STAGE IN *Pediculus humanus*

Age at hatching (days)	Number of eggs which hatched
5	7
6	3528
7	4259
8	7320
9	7470
10	1949
11	111
12	1

has been calculated as 5.23 days for the first, 3.40 days for the second, and 4.18 days for the third. Since the third molt gives rise directly to the adult, the mean duration of the entire larval period was 12.81 days, the standard deviation of which was only 0.67 days. In this group of larvae, the survival was 90.4 per cent, and the mortality was distributed rather uniformly over the three instars (table 3). In preparing the survivorship table, the 86.3 per cent survival figure was used, inasmuch as it was obtained from a larger series and from the same source that provided the data for eggs and adults.

ANALYSIS OF THE DATA

Despite the care with which the observations were obtained, it was necessary to make several assumptions in setting up the mortality and fecundity

TABLE 3

SURVIVORSHIP AND DURATION OF THE LARVAL PERIOD IN *Pediculus humanus*

Interval in days between hatching and		Number of larvae which molted	Number beginning larval life = 377
First molt	4	53	
	5	184	
	6	116	
	7	10	
			No. surviving first molt = 363
Second molt	8	150	
	9	186	
	10	18	
			No. surviving second molt = 354
Third molt	12	115	
	13	176	
	14	50	
			No. surviving third molt = 341

tables. In the first place, it was assumed that all of the eggs laid were fertile and that failure to hatch was due to embryonic mortality. It was impossible to determine time of death in the egg stage, and the total mortality for this phase of the life cycle was therefore assumed to be distributed evenly over the entire period. Similarly, the total larval mortality has been apportioned equally to each day of the larval period, since the daily mortality is not known; that such regularity in deaths probably does occur is suggested by the observed uniformity in mortality for each of the three instars. The sex of individual lice was readily distinguishable immediately after emergence as adults but could not be determined in the egg or larval stages, and it was therefore assumed that the mortality rates in these stages were equally applicable to males and to females. Finally, the assumption has been made that half of the eggs produced were female and half were male. Buxton (1939) notes that the sex ratio of the offspring of particular pairs of lice is often far from equality and that unisexual families have sometimes been reported, but there was no evidence of markedly uneven sex ratios in the many laboratory populations reared by us.

The analysis of the data follows that of Birch (1948) and Leslie and Park (1949) except that, because of the relative shortness of the life span and the fact that daily records were available, it proved unnecessary to adopt some arbitrary, larger unit for age grouping, a procedure which sacrifices a certain amount of possibly useful information. Table 4 presents the calculations. The life-table (l_x) function, shown in column 2, indicates the probability at birth of a female being alive at age x, when l_0 is taken as unity. The maternal frequency (m_x), given in column 3, shows the average number of female eggs produced per day by a female alive aged x. The sum of this column, 113.799, represents the gross reproduction rate, or the average number of daughter eggs that it is expected would be produced by a female living throughout the entire reproductive period. The average daily output of daughter eggs by such a female is 2.53. The sum of the fourth ($l_x m_x$) column, 30.930, is the net reproduction rate, R_0. It indicates that one female egg alive would on the average be replaced by approximately 31 live daughter eggs, that is, there is a 31-fold increase per generation.

The intrinsic rate of increase, r, was calculated by the method also employed by Birch and by Leslie and Park, which is reasonably accurate for comparatively low values. This method involves the use of trial values of r to find that value which will satisfy the equation $\Sigma e^{-rx} l_x m_x = 1$. Column five of table 4 shows the values of e^{-rx} when $r = 0.111$. With this value of r, the summation of $e^{-rx} l_x m_x$ for each age in which $m_x > 0$, shown in column six, proved to be 1.0027, a reasonably good approximation to the formula. (At $r = 0.112$, this summation was 0.9745.) The intrinsic rate of increase of *Pediculus humanus*, estimated from the data given above, may therefore be taken to be $r = 0.111$ per day. The mean length of a generation, given by $T = \log_e R_0 / r$, was 30.92 days. A population increasing at the rate of 0.111 per individual per day would double in numbers every 6.24 days.

TABLE 4

LIFE TABLE, FECUNDITY, RATE OF INCREASE, AND STABLE AGE DISTRIBUTION OF *Pediculus humanus*

1	2	3	4	5	6	7	8	9
x	l_x	m_x	$l_x m_x$	e^{-rx} (r = .111)	$e^{-rx} l_x m_x$	$e^{-rx} l_x$	Stable age dist.	Per cent of total population, stable age dist.
				Egg				
0.0	1.000			1.0000				
0.5	.993			.9460		.9394	118.6	
1.5	.978			.8466		.8280	104.5	
2.5	.964			.7577		.7304	92.2	
3.5	.949			.6781		.6435	81.2	
4.5	.935			.6068		.5674	71.6	67.88
5.5	.920			.5431		.4996	63.1	
6.5	.906			.4860		.4403	55.6	
7.5	.891			.4350		.3875	48.9	
8.5	.877			.3893		.3414	43.1	
				Larva				
9.5	.868			.3484		.3024	38.2	
10.5	.859			.3118		.2678	33.8	
11.5	.850			.2790		.2372	29.9 I	15.18
12.5	.840			.2497		.2097	26.5	
13.5	.831			.2235		.1857	23.4	
14.5	.822			.2000		.1644	20.8	
15.5	.813			.1790		.1455	18.4 II	6.99 26.43
16.5	.804			.1602		.1288	16.3	
17.5	.795			.1434		.1140	14.4	
18.5	.786			.1283		.1008	12.7	
19.5	.776			.1148		.0891	11.2 III	4.26
20.5	.767			.1027		.0788	9.9	
21.5	.757			.0919		.0696	8.8	
				Adult				
22.5	.749	0.934	0.700	.0823	.0576	.0616	7.8	
23.5	.748	1.628	1.218	.0736	.0897	.0551	7.0	
24.5	.729	2.212	1.613	.0659	.1063	.0480	6.1	
25.5	.718	2.363	1.697	.0590	.1001	.0423	5.3	
26.5	.700	2.527	1.769	.0528	.0934	.0369	4.7	
27.5	.678	2.733	1.853	.0472	.0875	.0320	4.0	
28.5	.665	2.617	1.740	.0423	.0735	.0281	3.5	
29.5	.640	2.688	1.720	.0378	.0651	.0242	3.1	
30.5	.609	2.825	1.720	.0339	.0582	.0206	2.6	
31.5	.579	2.687	1.556	.0303	.0471	.0175	2.2	
32.5	.553	2.877	1.591	.0271	.0431	.0150	1.9	
33.5	.511	2.389	1.221	.0243	.0296	.0124	1.6	
34.5	.469	2.649	1.242	.0217	.0270	.0102	1.3	
35.5	.439	2.670	1.172	.0194	.0228	.0085	1.1	
36.5	.397	2.817	1.118	.0174	.0195	.0069	0.9	
37.5	.356	2.923	1.041	.0156	.0162	.0055	0.7	
38.5	.328	3.029	0.994	.0139	.0138	.0046	0.6	
39.5	.299	2.737	0.818	.0125	.0102	.0037	0.5	
40.5	.267	2.940	0.785	.0112	.0088	.0030	0.4	
41.5	.261	2.721	0.710	.0100	.0071	.0026	0.3	
42.5	.235	2.835	0.666	.0089	.0060	.0021	0.3	
43.5	.216	2.539	0.548	.0080	.0044	.0017	0.2	
44.5	.189	2.640	0.499	.0072	.0036	.0014	0.2	5.69
45.5	.178	2.303	0.410	.0064	.0026	.0011	0.1	
46.5	.161	2.176	0.350	.0057	.0020	.0009	0.1	
47.5	.136	2.208	0.300	.0051	.0015	.0007	0.1	

TABLE 4 (*continued*)

x	l_x	m_x	$l_x m_x$	e^{-rx} (r = .111)	$e^{-rx} l_x m_x$	$e^{-rx} l_x$	Stable age dist.	Per cent of total population, stable age dist.
				Adult				
48.5	.125	2.371	0.296	.0046	.0014	.0006	0.1	
49.5	.112	2.380	0.267	.0041	.0011	.0005	0.1	
50.5	.101	2.708	0.274	.0037	.0010	.0004		
51.5	.089	2.394	0.213	.0033	.0007	.0003		
52.5	.072	2.434	0.175	.0030	.0005	.0002		
53.5	.051	2.722	0.139	.0026	.0004	.0001		
54.5	.036	2.895	0.104	.0024	.0002	.0001		
55.5	.033	1.824	0.060	.0021	.0001	.0001		
56.5	.021	2.636	0.055	.0019	.0001	.0000		
57.5	.017	5.056	0.086	.0017	.0001			
58.5	.015	4.000	0.060	.0015	.0001			
59.5	.015	1.688	0.025	.0013	.0000		0.1	
60.5	.011	2.833	0.031	.0012				
61.5	.010	2.200	0.022	.0011				
62.5	.010	2.200	0.022	.0010				
63.5	.009	1.500	0.016	.0009				
64.5	.008	2.125	0.017	.0008				
65.5	.006	2.166	0.013	.0007				
66.5	.002	2.000	0.004	.0006				
67.5	.000	0.000	0.000					
Sum		113.799	30.930		1.0027	7.9204	1000.0	100.00

Leslie and Park point out that in the case of species having a large relative rate of increase, calculation of the stable age distribution may have little meaning, since the great increase of density that would normally occur in a limited environment would certainly alter rapidly the age-rates of death and reproduction. The material necessary for such a computation, that is, a knowledge of the survivorship (l_x) curve throughout the entire life span, was already at hand, however, and we have made the calculation in order to compare the type of stable age distribution for *Pediculus humanus* with those published for other insects. Values for $e^{-rx} l_x$ calculated for each age group are shown in column seven of table 4. The proportion of the total $e^{-rx} l_x$ value represented by each age group and expressed in terms of 1000 females living is then given in column eight. In summary, this stable age distribution consists of 5.69 per cent adults, 26.43 per cent larvae, and 67.88 per cent eggs. These values approximate those obtained for Calandra (Birch, 1948) and Tribolium (Leslie and Park, 1949).

DISCUSSION

The intrinsic rate of increase of a population has been defined as the rate of increase per head under specified physical conditions and when the possible effects of increasing density do not need to be considered (Birch, 1948). For a given species, the intrinsic rates of increase under different sets of conditions should provide measures of the relative favorability of the respective environments. For different species, on the other hand, a compari-

son of intrinsic rates of increase obtained under different physical conditions may be extremely misleading (Leslie and Park, 1949). Nevertheless, there is presumably an upper limit to the intrinsic rate of increase for each species, and these maximal values of r should offer a basis for comparisons when one is not primarily interested in the specific conditions of the environments. If the optimal conditions for a species be defined as those under which the maximum rate of increase can be realized, and if the intrinsic rates of increase of two or more species have been measured under such conditions, then a comparison of r values should be significant regardless of what the actual physical environments were. In a laboratory experiment designed to determine the intrinsic rate of increase, it is probable that as nearly optimal conditions for development as possible will be provided, unless the experiment is also concerned with the effects of competition or some other density-dependent factor. It will be assumed in this discussion that the various values of r which are referred to were obtained under approximately optimal conditions and represent maximal or nearly maximal rates of increase.

In table 5 we have assembled those values of r which have been published for five laboratory-reared species (the short-tailed vole, *Microtus agrestis;* the laboratory rat, *Rattus norvegicus;* the flour beetle, *Tribolium castaneum;* the rice weevil, *Calandra oryzae;* and the human louse, *Pediculus humanus*), together with information pertaining to associated aspects of population growth and development. These r values vary from 0.01 to just over 0.10, a seemingly rather narrow range. Indeed, the bacteriophage T_2 of *Escherichia coli,* which can increase on the average 120-fold every 21 minutes (Delbrück, 1946), has an r value of 328, and it is certain that such a slowly developing species as the 17-year cicada has an r less than 0.001. The range of r values calculated from experimental life-table data is unlikely ever to be more than a small fraction of the total r spectrum. This indirect method of determining the intrinsic rate of increase is mathematically laborious and is resorted to primarily when the life span of the species is so long and the space requirements of the individual organisms are so great that direct observation is impracticable or impossible. Nevertheless, the small range of values of r which have thus far been obtained by this method provides a number of interesting comparisons.

Human rates of increase are not entirely comparable with those in table 5, for it is exceedingly difficult to evaluate and to remove the effects of density in the case of human populations. There are no data for man that are reliably density-independent, and we have therefore refrained from including human material in the comparison.

The two rodents differ sharply from the three insects in the order of magnitude of the intrinsic rate of increase, that of the latter being approximately eight times as great as that of the former. Much of this difference is evidently related to differences in the times for development, represented in table 5 by the mean length of a generation in days. It is clear that, other things being equal, growth rates and times for development, as defined above, are inversely linear. Therefore a species which lengthens its period of

TABLE 5

COMPARISON OF DATA PERTAINING TO RATE OF INCREASE FOR VARIOUS SPECIES OF RODENTS AND INSECTS

	Species	Intrinsic rate of increase (r/day)	Mean length of generation in days (T)	Net reproduction rate (R_O)	Time required to double population (days)	Source of data
Rodents	*Microtus agrestis*	0.0125	141.75	5.904	55.44	Leslie and Ranson, 1940
	Rattus norvegicus	0.0147	217.57	25.66	47.14	Leslie, 1945
Insects	*Tribolium castaneum*	0.101	55.6	275.0	6.86	Leslie and Park, 1949
	Calandra oryzae	0.109	43.4	113.56	6.36	Birch, 1948
	Pediculus humanus	0.111	30.92	30.93	6.24	Evans and Smith, 1952

development may pay a price for doing so in a corresponding decrease in population growth rate. Unless increased time of development is accompanied by compensatory changes, such as lowered mortality or increased fecundity, the reduced growth rate may not be sufficient to meet the exigencies of the environment. A species with such a deficiency will become extinct.

If all of the difference in the five intrinsic rates of growth was explainable by the mean generation periods, their products should be constant. The actual values of these products, however, are as follows:

Microtus agrestis	1.772
Rattus norvegicus	3.198
Tribolium castaneum	5.616
Calandra oryzae	4.731
Pediculus humanus	3.432

These values are the natural logarithms of the net reproduction rates, which are also shown in table 5, and they suggest a further relationship affecting the intrinsic rate of increase.

The net reproduction rate as given here indicates the number of female eggs or young which would be expected under optimal conditions to replace a given female egg or young of the previous generation. A comparison of the net reproduction rates given in table 5 shows that at maximum rates of increase a female Rattus is replaced by more than four times as many individuals as is a female Microtus. The position of the graminivorous insects, with their high reproductive rates, is not surprising. The astonishing species is the louse, which under optimal conditions is replaced by approximately the same number of offspring as the rat.

A species that is well adjusted to its natural environment may be expected to maintain rather constant numbers if the environment remains constant over a reasonably long period of time. In such a case, the factors which repress population growth would counterbalance the maximum capacity for growth. It may be assumed, as a working hypothesis at least, that the maximum growth capacity of a species is the result, not of an accident, but of evolutionary process and that it represents an adjustment to the long-term requirements of its natural habitat. The implications here are (1) that the maximal value of r is held above a critical level by natural selection, and (2) that higher values, for which there would be no net positive selection, are reduced through the general economy of the organism. Under natural conditions, then, the intrinsic rate of increase will tend to be reduced to zero and the net reproduction rate to 1.0. From the population point of view, the size of the maximal r, or its distance from zero, should therefore reflect the degree to which the natural environment departs from conditions conducive to greatest growth. Similarly, the magnitude of R_O measured under conditions which are optimal for maximum growth should indicate the rigorousness of the natural environment in terms of individual replacement.

To refer again to the rates in table 5, and remembering the assumption that these represent maximal or nearly maximal rates of growth, a few further

comparisons can be made. Both in terms of the population and in terms of the individual, the two rodent environments would seem to be much closer to optimal than the three insect environments. Within each of the two groups, however, relations are less clear.

From the two rodent values of r, it is seen that the natural environments of the vole and the rat are nearly alike in their distances from optimal, with that of the vole population slightly closer than that of the rat population. The two values of R_O, however, suggest that the likelihood of a vole being born and becoming mature in its natural environment is much closer to expectancy under optimal conditions than is that of a rat. Since R_O can be reduced to 1.0 not only by increased mortality but also by reduced fecundity, the comparison here concerns the likelihood of coming to exist at all as well as of survival through maturity. In view of the apparent fecundity of rats under natural conditions, it is evident that their survival must be relatively poor.

Among the three insects, the maximal intrinsic rates of growth are much alike, suggesting equivalent deviations of the natural environments from the optimal environments in terms of population survival. In terms of the individual, however, the three natural environments appear to be very different. A comparison of the R_O values for the two graminivorous species indicates that the "distance" of the natural environment from optimal conditions is more than twice as great in *Tribolium castaneum* as in *Calandra oryzae*. There is a large discrepancy between the R_O values of these two species and that of the louse, which seemingly belongs to another order of magnitude altogether. The low value of the net reproduction rate in *Pediculus humanus* is indeed surprising, for a high rate is usually associated with the parasitic mode of life. The indications are that the individual louse has a much better chance of surviving and propagating its kind than the individual flour beetle or rice weevil, and that its natural environment is remarkably secure.

SUMMARY

Data on mortality, fecundity, and duration of life history stages were obtained for lice kept in uncrowded colonies at a temperature of approximately 30° C. and at a relative humidity which varied between 30 and 55 per cent. Under these conditions the intrinsic rate of natural increase, that is, the rate of increase per head in a population which has attained a stable age distribution, was calculated to be 0.111 per day for *Pediculus humanus*. In this population, one female egg alive would on the average be replaced by 30.93 live daughter eggs, and 6.24 days would be required for the population to double in numbers. A comparison of vital statistics with those already published for two species of graminivorous insects and two species of rodents, all of which are assumed to have been obtained under conditions conducive to maximum rates of growth, suggests that the natural environment of the individual human louse is a remarkably safe one.

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SUPERFETATION IN THE RANCH MINK[1]

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Litters of young part of which are sired by one male and part by another male are not uncommonly encountered among litter-bearing mammals where matings to different males take place within a single "heat" period. An exception to this general observation seemed to have been provided by the occasional mink rancher who reported litters of kits containing offspring from different males during the early part of the past decade, after the dominant color phases came to be bred in large numbers. Such "split" litters have been noted following two matings, most commonly spaced seven days apart, but in a few cases as many as 20 days elapsed between matings.

The breeding season of the ranch mink (*Mustela vison* sp.) is limited to a single period of the year, extending from the first days of March through the first week of April. It became apparent in the early days of domestication that the reproductive processes in the female mink exhibited several remarkable features. Some individuals accept the male only once, others two or three times with several days intervening between matings, and occasional females take the male at practically every opportunity within the breeding season. The length of the gestation period is considerably more variable than in any of the other species, ranging from 38 to 76 days for once mated females with the average being about 50 days.

Hansson (1947) has shown that the mink is a non-spontaneous ovulator, and that ovulation occurs 36–37 hours after mating. He also discovered that mink regularly ovulate a second time in the same season, provided a minimum of six days is allowed between matings. This author has given an exhaustive review of the literature relating to reproduction in the mink and other Mustelids. No attempt at duplication will be made here.

Several possibilities may be considered in an attempt to explain the occurrence of split litters: (1) a mink may ovulate twice and eggs from both ovulations contribute to the same litter; (2) ovulation may occur at the second mating, but sperm from the first mating survive and may be able to compete successfully with fresh sperm in fertilization; (3) ovulation may occur at the first mating, but for some reason some of the eggs are not fertilized at this time and are still capable of being fertilized at the second mating.

Two experiments designed to shed further light on the physiology of reproduction in the mink were begun at this station in 1947. The first was concerned with whether or not split litters could be produced, and if so

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what proportion of all litters from twice-mated females contained kits from both males. The second was made to investigate the possibilities of two separate ovulations contributing to a single litter.

EXPERIMENTAL TESTS—1

A method of experimentally testing the possibility of two males jointly siring a litter is to mate dark (wild type) females, at appropriate intervals, to dark (wild type) males and to males homozygous for a dominant gene affecting coat color. Two males of the royal silver color phase were available which progeny tests had indicated to be homozygous for the mutant gene s^R, thus making them ideal subjects for double mating experiments. The complete history of these mink has been presented elsewhere (Shackelford, 1949).

Dark females were exposed to dark males on the seventh day after a first mating to one of the royal silver males, and vice versa. An attempt was made to have approximately half of the first matings made to the royal silver males, and half to dark males. Females which would not re-mate by the tenth day, or failed to produce a litter have not been included in the results presented here.

Results: The results of mating 27 dark females to two genetically differentiated males are shown in table 1. The male used for the second mating sired all kits of each litter in 21 cases; 2 litters were sired entirely by the first used male; 4 litters contained a single kit from the first male, the other kits resulting from the male used for the second mating. No more variation was evident in the size or stage of development among the kits in the split litters than in those litters which were sired entirely by a single male.

It seems possible for split litters to be a fairly common occurrence in the mink since in this experiment 14.8 per cent of the litters contained kits sired by both males. An error of approximately 8.9 per cent would be expected if it were assumed that all kits in a litter were sired by the second male.

TABLE 1

RESULTS OF MATING MINK FEMALES TO TWO GENETICALLY DIFFERENTIATED MALES (1947–48)

Interval between matings (days)	Number of litters	Number of litters containing kits from mating			Number of kits from mating	
		1	2	1 and 2	1	2
6	16	0	14	2	2	72
7	6	1	4	1	5	24
8	2	0	1	1	1	8
9	3	1	2	0	3	9
Totals	27	2	21	4	11	113
Proportion of split litters				14.8 per cent	....	....
Proportion of kits from the first used male					8.9 per cent	

EXPERIMENTAL TESTS—2

Experiment 1 demonstrated the possibility of two males jointly siring a litter of young when the interval between matings is 6–9 days. The results of this experiment did nothing, however, toward answering the question as to which one or more of the possibilities enumerated earlier in this paper might be involved in the production of split litters.

Hansson's evidence for the conclusion that mink may ovulate twice in a breeding season was based on finding two sets of corpora lutea which histologically appeared to be of different ages at the autopsy of females that had been twice mated. Experiment 2 was also designed to determine whether or not two ovulations may occur, using a system of marking the first set of ovulation points and being able to recognize them with certainty after the female had been mated a second time.

Abdominal surgery often leads to the death of the individual if the skin wound is closed by the use of ordinary sutures since the mink may continuously re-open the wound by chewing away the stitches. When the type of small metal clip employed for closing skin incisions in other small animals is used, however, the wound will not be molested. No casualties have resulted from closing the skin opening in this manner, and all wounds healed satisfactorily.

During the 1948 breeding season seven females were mated as indicated in table 2, and a minimum of 48 hours allowed to elapse before the females were opened for examination. At laparotomy the right ovary was removed for sectioning if necessary, but there proved to be no difficulty in determining the number of ovulation points in an intact ovary. The left ovary remained undisturbed except that an opening was made in the bursa large enough to expose it, and the ovulation points were counted and marked by puncturing them with a small hypodermic needle which had been dipped in India ink. The opening in the body wall was sutured with *oo* size cat gut and the skin incision closed by use of wound clips.

Each female was placed with a male for a second mating on the seventh day following the first mating, and those that did not copulate were tried for two additional days. A minimum of 48 hours was allowed to elapse before the females which had mated a second time were killed, and the left ovary examined for both old (marked by India ink) and new (ovulation points) sets of corpora lutea.

Results: Six of the seven females mated a second time as shown in table 2. All had ovulated twice or were in the process of ovulating a second time at autopsy. Since some of the India ink particles remained at each site of injection, gross examination of the ovary was sufficient for recognition of the set of corpora lutea resulting from the first mating in all cases except for ♀ 32 (figure 1 B, upper); sectioning was necessary to locate one of the marked corpora that had receded beneath the ovarian surface in this individual. The number of ovulation points in the left ovary resulting from the second mating were as easily determined as those had been in both the right and left ovaries at laparotomy following the first mating.

TABLE 2

OVARIAN CHANGES IN THE MINK FOLLOWING TWO MATINGS

Female number	Interval between matings (days)	Right ovary		Left ovary					
				Results of first mating			Results of second mating		
		Hours removed after first mating	Number ovulation points	Number ovulation points marked	Number marked corpora (autopsy)	Number blastocysts in uteri (autopsy)	Hours removed after second mating	Number new ovulation points (autopsy)	No. eggs recovered from oviducts (autopsy)
37	7	51	5	6	6	0	48*	5	....
38	7	48	6	5	5	0	52	3	3
33	6	48	6	5	5	0	65	7†	....
43	7	48	8	3	3	0	95	8	....
44	7	48	4	5	5	6	74	6	2
32	7	75	5	4	3‡	0	53	8	4

*Female found dead 48 hours after second mating.
†Six eggs in large follicles plus one egg being ovulated.
‡Fourth corpus found after sectioning.

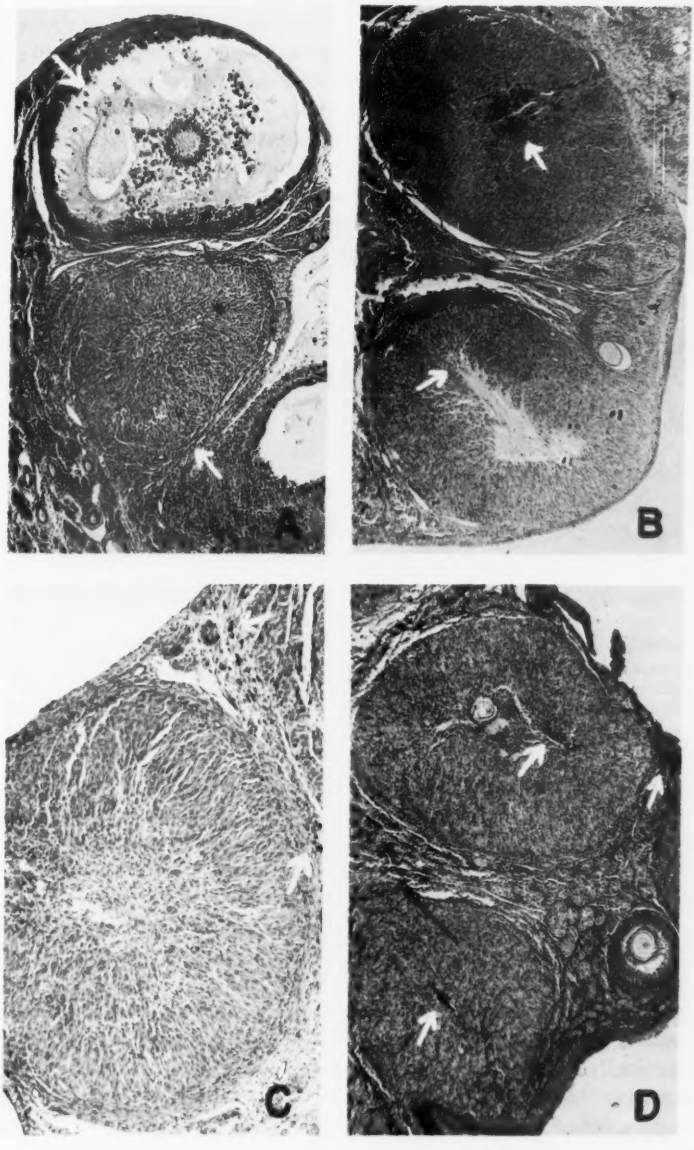

FIGURE 1. Cross sections of some ovaries from twice mated mink. White arrows indicate areas retaining India ink particles in B (upper), C and D.

A. (× 40) Upper, a mature follicle ready for ovulation. Lower, a corpus luteum resulting from an ovulation approximately 8 days prior to autopsy.

B. (× 40) Upper, a marked corpus luteum from the first ovulation of the season. Lower, a corpus luteum resulting from the second mating which followed the first mating by 8 days.

C. (× 75) Occasionally the marking needle failed to hit the target as indicated by the ink particles in the connective tissue surrounding the corpus luteum.

D. (× 40) Two well-marked corpora lutea.

Female No. 37 was found dead approximately 48 hours after the second mating. Whatever the cause of death, it had not prevented a second ovulation. Although 65 hours had elapsed since the second mating, at autopsy ♀ 33 (figure 1 A) appeared to be ovulating. One egg was oozing from its follicle and six others appeared to be ready for extrusion.

The uteri of all females were flushed with normal saline in an attempt to recover blastocysts from the first mating, on the assumption that developing eggs reach the uteri by the seventh day after mating. No blastocysts were found in five females, but six were recovered from ♀ 44, leaving three

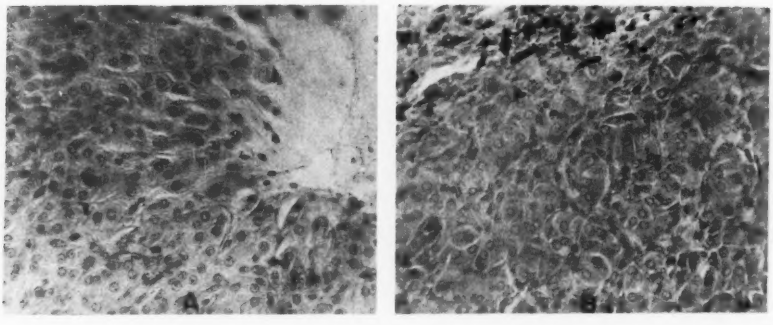

FIGURE 2. Enlargements of the areas indicated by the white arrows in each of the corpora lutea shown in figure 1 B (× 450). A. Corpus luteum resulting from second mating which occurred 53 hours prior to autopsy. B. Corpus luteum from first mating which took place approximately 10 days before autopsy. Note the India ink particles.

eggs unaccounted for on the basis of the nine ovulation points seen at laparotomy after the first mating.

The oviducts of three females were removed, placed individually in watch glasses containing normal saline and manipulated with dissecting needles under binoculars in an effort to recover eggs from the second mating. Eggs were found in all cases; in ♀ 38, an egg for each ovulation point was obtained; two out of a possible six were found in ♀ 44, and four out of a possible eight for ♀ 32.

Attention is drawn to the fact that this experiment was carried out after Hansson's similar experiment was finished, but his published results were not generally available at this time and were unknown to workers in this laboratory. Therefore, Experiment 2 should be considered an independent corroboration of the Swedish workers' findings. Although the proof of double ovulation in the present experiment depended (by plan) upon marking the first set of corpora lutea for certain identification after the second ovulation, comparison of comparable figures in Hansson's paper with those in this paper (figure 2) shows that the corpora in both experiments have the same appearance as would be expected.

DISCUSSION

Superfetation is a subject to which considerable attention has been directed from time to time for more than a century. King (1913) appears to be the first to make a clear distinction between superfetation and superfecundation, defining superfecundation as the fertilization by successive matings of ova belonging to the same period of ovulation, and superfetation as the fertilization of ova of different periods of ovulation. Her definition will be accepted for the discussion in this paper.

Two females had a single blastocyst in the uterus along with eggs in their oviducts in Hansson's group of 27 females that had been twice mated. If it be assumed that these blastocysts were capable of normal development, then a mechanism for true superfetation in this species had been discovered. There would seem to be no question that two separate periods of ovulation are involved here since Hansson found that females could not usually be made to ovulate again sooner than the sixth day after a previous mating.

The 1947–48 matings (Experiment 1) showed that two sires may contribute to one litter. Combining the results of the 27 matings, all of which had a 6–9 day mating interval, 8.9 per cent of the kits were sired by the first used male. Johansson and Venge (1951) bred females at 6–19 day intervals to appropriately marked males and have reported that from 52 litters produced, 14.0 per cent of the offspring were sired by the first male.

There is a noticeable discrepancy between 8.9 per cent and 14.0 per cent, but it should be recalled that the breeding intervals are not comparable in the two experiments; more than half of the matings reported by Johansson and Venge represent females which had a mating interval of 10–19 days. When only those matings from the 6–9 day interval group (25 matings) are considered for comparison with the data presented here (27 matings), then the proportion of kits from the first male is 12.5 per cent. Further comparison of the two experiments shows that there is even closer agreement between them if it is noted that the Swedish workers found 8.0 per cent of the young from the first male in the 6–7 day interval group (10 matings), and 6.8 per cent is the comparable figure from table 1 (22 matings).

A plausible explanation of split litters in the mink would seem to be that two separate ovulations contribute to the same litter as suggested by the work of Hansson and the data presented in table 2. This view is particularly attractive when it is considered that delayed implantation is thought to occur frequently in the mink, and to account for much of the variability in length of the gestation period in this animal. Although the two other possibilities enumerated are not ruled out, it would appear unlikely that they contribute to the production of split litters.

Experimental superfetation has not been demonstrated in other mammals so far as is known to this writer, and thus in this aspect of reproduction the mink appears to be unique among the species investigated. It would seem reasonable to conclude that superfetation is a normal and regular phenomenon

in the mink with appropriate mating intervals, but is not the rule since the greater proportion of the offspring result from the last mating, most of the blastocysts from the former mating or matings failing to persist.

There is a considerable body of experimental evidence indicating the improbability of the existence of the type of superfetation found in the mink in mammals where there is normally no delayed implantation. A series of reports by Casida et al. (1940, 1943), Boyarsky et al. (1947), Murphree et al. (1944, 1947, 1951), Tanabe et al. (1949) and Black et al. (1951), demonstrate, among other things, that eggs ovulated experimentally during the luteal phase of the sexual cycle in a number of animals (cow, rabbit, ewe, sow) are not readily fertilized. Boyarsky et al. state: "The data furnish evidence that the condition of infertility in animals that are treated during pseudopregnancy is not one which arises at the inception of pseudopregnancy, but is built up gradually over a period..." and "suggests a gradual elaboration of an endocrine substance such as might come from a developing corpus luteum."

The preceding paragraph implies that the mink may be different from some other species in that delayed implantation in this Mustelid can signify corpora lutea which are not producing sufficient quantities of progesterone to bring about early implantation, and that this low level of progesterone also allows a second estrus and the ovulation of a second set of eggs capable of normal development. There would appear to be no evidence against this consideration. Such data as have been presented lend credence to this interpretation: the experiments of Johansson and Venge and the data in table 1 indicate that the longer the interval between two ovulations, the greater the chances of survival of the embryos from the first ovulation. This can be interpreted in at least two ways. The longer the corpora from the first ovulation have been functioning, (1) the greater is the chance that the last ovulated set of eggs is retarded in development, this view assuming there is then some sort of competition between the two sets of blastocysts; or, (2) the more likely it is that there is a reduction in the number of eggs ovulated in the second set, those ovulated being fully capable of normal development, this view assuming that there is an upper limit to the number of embryos a female can support to term. There is no evidence from these experiments for either of these interpretations.

SUMMARY

1. Reports of "split" litters from commercial mink ranches suggested the possibility of superfetation in this species.

2. Data have been presented in which genetically differentiated males were mated to females at intervals of 6–9 days; 14.8 per cent of the 27 litters contained offspring from both males, and 8.9 per cent of the young were sired by the first used male.

3. By the use of a system of marking corpora lutea, it was determined that mink regularly ovulate a second time in the same breeding season if a 6–7 day interval is allowed between matings.

4. The presence of blastocysts in the uterus and fertilized eggs in the oviducts of the same individual, presumably from the first and second ovulations respectively, point to a mechanism by which true superfetation may occur.

5. Superfetation is apparently a normal and regular phenomenon in the mink under the conditions stipulated, although not the rule.

6. It is suggested that superfetation as it occurs in the mink is not likely to occur in animals in which there is normally no delayed implantation.

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A MUTABLE LOCUS IN WILD POPULATIONS OF HOUSE MICE

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In certain laboratory stocks of *Mus musculus* one locus has proved to be extremely mutable. This is the locus first marked by the Brachyury (short-tail) mutation $T/+$, and later by a series of mutations allelic to T, known as t-type mutations, which express themselves by producing taillessness in compound with T, viz. T/t^1-T/t^{12} (Dunn and Gluecksohn-Schoenheimer, 1950). Ten of these have been identified and studied and exceptions representing perhaps as many more alleles have occurred and been lost for one reason or another. The usual method of detecting these is by testing exceptional normal-tailled animals in the balanced lethal stock T/t^1 which usually breeds true to taillessness. Exceptions usually prove to be t^1t^n.

Although little is known about the manner of origin of such mutations, we have usually assumed that they are connected with some instability at this locus due to the presence of t^1 which since it also suppresses recombination in the vicinity of T appeared to be a sectional change, probably a short inversion (Jaffe, 1952).

In order to get further light on this interesting locus we have recently been testing members and descendants of wild populations and have found t-type alleles in the first population tested and in one animal from a second population.

The tests are easily made. Wild males are mated to Brachy $T/+$ females of an inbred laboratory stock. If tailless offspring appear, they are tested by known tailless individuals (T/t^1 or T/t^o). If the new tailless is T/t^n, only tailless (T/t) and normal (t^1/t^n) progeny appear from the test cross; if the new tailless is $T/+$ with a modifier at another locus, then in addition to tailless T/t^1 and normal $+/t^1$, Brachy offspring $T/+$ also appear.

The first tests gave the results shown in table 1. The ten males in this table were from a population of wild mice maintained by Dr. Howard A. Schneider of the Rockefeller Institute and kindly supplied by him. This population is descended from mice caught wild in suburbs of New York and in Philadelphia in 1943-4. It has since undergone moderate inbreeding although brother-sister matings have been avoided (Schneider, 1946). Of the ten males, two failed to breed, two gave only normal and Brachy offspring, one gave normal and Brachy and a single tailless son which on testing proved to be $T/+$. Of the remaining five, each produced several tailless offspring. Ten of these F_1 tailless animals from three of the wild males have been tested with the results shown in table 2. The absence of Brachy offspring indicates the most probable genotype of the F_1 tailless as T/t^w and of the wild parent as $+/t^w$ using t^w as a collective term for t-alleles

TABLE 1

RESULTS OF TESTING MEMBERS OF A WILD POPULATION BY BRACHY *T*/+

Wild parent	Offspring		
	Normal	Brachy	Tailless
♂ 4922	23	2	20
27	29	5	20
28	14	1	14
29	14	2	7
30	6	....	4
Total	86	10	65
♂ 4923	23	28	1*
4925	14	13	
4926	26	11	
4921	....	....	
4924	....	....	

*Progeny tested and shown to be *T*/+

found in the wild. If this is so then the test cross of heterozygous wild male by Brachy should produce the following results:

	Sperm	
	+	t^w
eggs +	normal	normal
T	Brachy	tailless

This is a ratio of .5 normal .25 Brachy .25 tailless. The departure of the actual ratio of Brachy to tailless (10:65) from the expected is highly significant. It probably indicates that the wild heterozygotes produce 5 to 6 times as many mutant (t^w) as normal (+) gametes. This excess in males

TABLE 2

RESULTS OF TESTING F_1 TAILLESS ANIMALS FROM TABLE 1 BY T/t^1 AND T/t^0 TAILLESS

Wild parent $+/t^w$	F_1 tailless parent T/t^w	Offspring		
		Normal	Brachy	Tailless
♂ 4922	♂ 4878	24		22
4922	5235	5		12
4927	5284	4		9
4927	5429	3		9
4927	5430	5		4
4927	5465	4		6
4929	5255	5		8
4929	5256	14		15
4929	5257	8		15
Totals		72		100

heterozygous for lethal *t*-alleles has been known previously (t^0, t^1, t^9, t^{12}—Dunn and Gluecksohn-Schoenheimer, 1950). It may be the significant factor in maintaining *t*-alleles, which are probably lethal, in wild populations.

It is not yet known whether all five of the wild males shown to be heterozygotes carry the same *t*-allele. Their descent from the same closed population makes it likely that only one mutant allele is involved. Other questions remaining to be tested are 1) lethality of the *t*-alleles from the wild; 2) relative viability of +/+ and $+/t^w$ members of wild populations.

A single female caught wild in Mystic, Connecticut, by Mr. W. C. Morgan, Sr., has when tested by a Brachy male, given two tailless and one Brachy offspring, indicating concealed heterogeneity at this locus in a second population.

SUMMARY

Of eight males from one wild population five proved to be heterozygous for an allele at locus *T*, giving tailless T/t^w when crossed with Brachy *T*/+ laboratory females. The segregation ratio $+:t^w$ in such wild males was $10+:65t^w$, indicating significant excess of the mutant allele. A single female from a second wild population was also $+/t^w$. The appearance of mutants at this locus in the first two wild populations tested is evidence that this locus is highly mutable in the wild.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

CONCERNING PATTERN FORMATION BY FREE-SWIMMING MICROORGANISMS

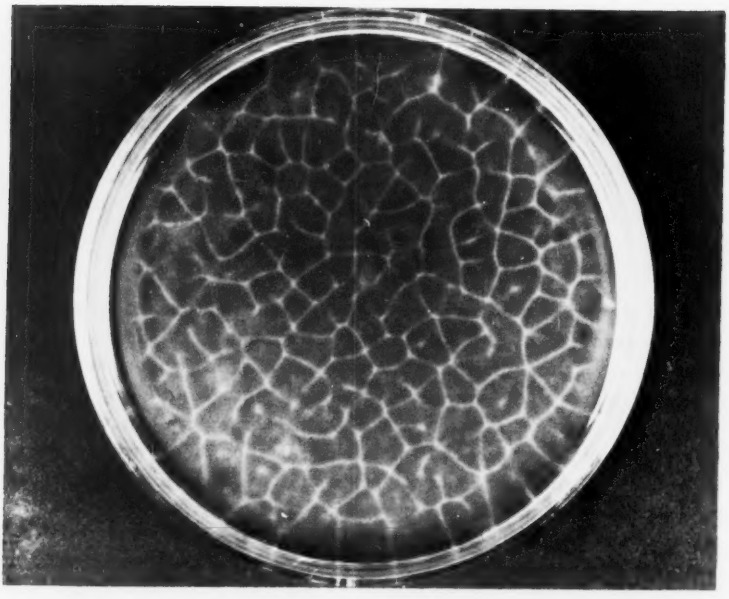
Several reports in the literature[1,2,5] have focused attention on an interesting phenomenon, viz., that motile organisms such as algae-zoospores and *Euglena gracilis* form distinct culture patterns. This phenomenon also occurs in cultures of the free-living ciliated protozoan, *Tetrahymena pyriformis,* but is not apparent in culture tubes unless very dense cultures are observed. Within a few seconds after shaking such tubes, irregular aggregation of the organisms is evident. This cultural behavior was observed in this laboratory a number of years ago when experimenting with optical density methods for the measurement of growth. It is essential, for this reason, to use extreme care in obtaining optical density measurements on these ciliates or they may be subject to considerable error. When the ciliates are grown in Roux flasks in which the medium may be up to 1½ cm. deep, the patterns are most striking. The results of a number of experiments designed to analyze this phenomenon are described below.

EXPERIMENTAL

Two photographs are shown, indicating variation noted under different conditions. The pattern lines consist of stratified columns of organisms, and as seen from the surface, they are extremely variable in shape. Although individual lines approach an equilibrium, each is actually in a state of dynamic equilibrium and the entire pattern is relatively fluid and actually changes in size and shape during a period of observation. Following careful addition of a small amount of a lytic and surface tension-depressing agent such as desoxycholate, a portion of surface of the culture is cleared enabling direct observation of the pattern in the interior of the medium. Study of the pattern is also facilitated by the addition of small quantities of particulate colored particles. Globular clusters in a high state of peripheral rotary motion, giving the cluster the appearance of a "boiling" mass, are seen. Each globule appears to be an entity within itself, in which the relatively few particles in the interior are rapidly migrating to the periphery, where they are caught up in a stream of organisms already concentrated there, hurled around the periphery and eventually scattered into the interior again to repeat the process. The velocity of organisms within this globule is far greater than that of an individual's rate of movement.

There is a critical depth of fluid below which no pattern formation occurs. We found this to be about 2 mm, as did Robbins[2] for Euglena. Below

a



b

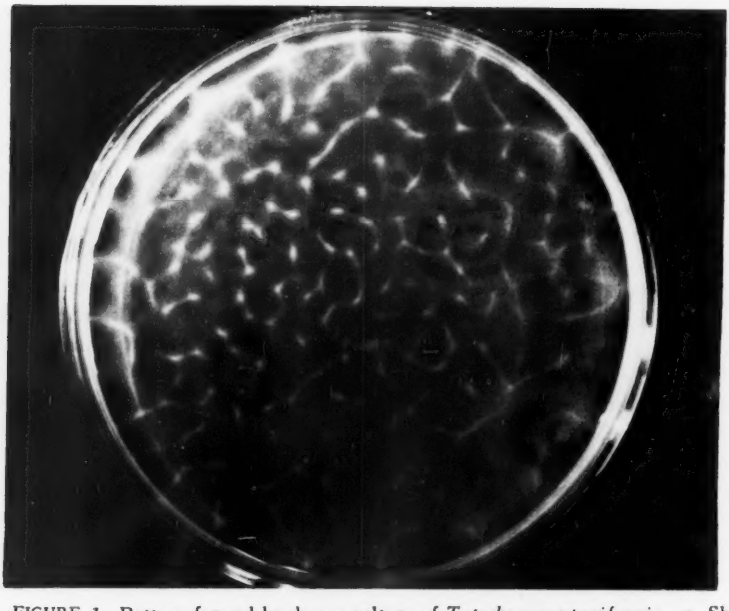


FIGURE 1. Pattern formed by dense culture of *Tetrahymena pyriformis*. a. Sharp pattern formed by young highly motile culture. Time of formation: 12 seconds. b. Diffuse pattern formed by old culture. Time of formation: 16 seconds.

this threshold, the organisms merely tend to concentrate at certain points, as also occurs below the critical concentration of organisms. Since the phenomenon appeared to be dependent on the concentration of organisms, a series of dilutions was prepared from a dense 3-day culture containing, by determination of most probable number[4] 1,300,000 viable ciliates per ml. Twenty-ml volumes of organisms as follows were prepared: 100, 75, 50, 25, 12.5 and 5. Pattern formation occurred within 12 seconds at 33°C, unless the dilution was less than 1:4. At 1:8, a longer period was required, and no patterns were noted in higher dilutions. In other words, no patterns were formed when the concentration was below 150,000 organisms/ml. Whenever density of a culture exceeds this value, age appears to exert no influence on pattern formation, and only slight influence on pattern shape, which is probably due to lowered motility.

Inasmuch as Robbins[2] had suggested that pH was a critical factor, a series of buffered media was prepared as follows: pH 3.0, 4.0, 4.5, 5.2, 6.6, 7.0, 7.8, 8.2 and 8.6, and inoculated with ciliates to obtain a concentration of approximately 500,000/ml. Pattern formation occurred within 12 seconds at 33°C, within the range pH 4.5–8.2, inclusive. No differences were noted either in speed of formation or in type of pattern formed. Organisms were inactivated at levels below pH 4.5. There was distinct inhibition of pattern formation at pH 8.6, at which H-ion concentration Tetrahymena is less active. We failed to note inhibition at physiological pH levels, as Robbins observed for Euglena.

A series of NaCl solutions was prepared to determine whether osmotic pressure would have any effect on this phenomenon. Pattern formation was not inhibited up through 0.075 M NaCl, but was markedly inhibited at 0.15 M, and the organisms agglutinated rapidly at higher concentrations.

Although Tetrahymena is highly aerobic, it will live and remain motile for a considerable time under anaerobic conditions. Experiments showed that with organisms placed under strictly anaerobic conditions (H_2 gas) pattern formation was not inhibited until motility itself was inhibited. Likewise, the addition of a reducing agent, thioglycollic acid, at pH 7.0, inhibits pattern formation only after motility is reduced.

The action of physical agents, such as ultraviolet radiation, in inhibiting pattern formation, is again directly correlated with its action on motility. Irradiation from 1–6 minutes failed to inhibit either pattern formation or motility, whereas higher dosages increasingly inhibited both. At 18 minutes, approximately one-half the organisms were no longer motile and pattern formation was completely inhibited.

Since pattern formation is indirectly the result of the combined motilities of a large number of organisms within a suitable area, any treatment that would alter individual motility should directly affect the total phenomenon. An inhibitor of ciliary motion, Parathion,[3] was found to inhibit pattern formation directly as a function of concentration. An inert substance, methyl cellulose, added in various concentrations to render suspensions more viscous, increased the pattern formation time from 8 seconds in the control suspen-

sion to 10 seconds with 2 per cent, 16 seconds with 4 per cent, and 20 seconds with 6 per cent methyl cellulose.

An experiment was carried out, using buffered cultures in the pH range previously described, at temperatures of 33, 23, 19, 10 and 7°C. Hydrogen-ion concentration exerted no influence at these temperatures, *per se,* but temperature per *se,* however, had a pronounced effect. The rate of pattern formation decreased in direct proportion to decrease in temperature. Furthermore, the pattern became progressively finer, *i.e.,* contained smaller facets.

It is observed that pattern formation results only when there is a minimum concentration of organisms in a suitable (non-confining) area and when these organisms possess a minimum threshold of motility. These conditions are essential to create the required turbulent-like motion which results in this unique pattern.

The course of events following external disturbance of a heavy suspension may be divided into 3 phases: (1) turbulence formation when the culture is shaken with resulting eddies and eddies within eddies, which results in an aggregation of organisms completely independent of their individual motility, (2) the intermediate, short-lived period of random swimming immediately following cessation of gross motion within the fluid, and (3) the formation and rapid organization of the pattern, in which large numbers of organisms synchronize their movements to create the characteristic "boiling" pattern in which the rotatory velocity within each globule far exceeds individual velocity. The self-created turbulent-like motion by motile organisms, described in only a few instances, represents an exceedingly complex phenomenon. Turbulence, uncomplicated by individually motile particles, is itself complex and poorly understood.

SUMMARY

The action of several agents and conditions upon the pattern formation observed in cultures of motile microorganisms has been studied using protozoa, viz., *Tetrahymena pyriformis.* A general description of the macroscopic and microscopic characteristics of the pattern and of its formation and organization was given. It was shown to be dependent upon both concentration of ciliates and the depth of the suspension. Hydrogen-ion concentration, *per se,* had no influence. Anything which decreases motility, e.g., low temperature, high osmotic pressure, anaerobic conditions, ultraviolet radiation, Parathion, methyl cellulose and advanced age, directly decreases the rate of pattern formation, and the sharpness of its facets once formed. Once the number of motile cells in a culture falls below the critical concentration, it will no longer form a pattern.

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MUTATION TESTS ON *DROSOPHILA MELANOGASTER* WITH AQUEOUS SOLUTIONS OF FORMALDEHYDE

Formaldehyde, when mixed with the food of Drosophila, produces mutations in male germ cells.[1,2,3,4] Used as vapor on larvae or adult flies, it proved ineffective.[5] This suggested the idea that the effective mutagen in the feeding experiments is not formaldehyde itself, but some compound formed between it and a constituent of the food. Attempts by Dr. Moser in this institute (see 6) to produce such a compound through formalin treatment of casein were, however, not successful. Although casein which has been so treated and has then been washed free of adsorbed formaldehyde is decidedly mutagenic for Drosophila larvae, this effect is lost when washing is continued long enough to remove the reversibly bound formaldehyde. It thus seems possible that also in the feeding experiments free formaldehyde is the actual mutagen, which is gradually released in small and only mildly toxic doses from reversible compounds with food constituents, and that the ineffectiveness of the vapor method is due to its high toxicity. If this is the case, milder methods of treatment with free formaldehyde should be mutagenic, and this has, in fact, been reported by various authors using aqueous solutions.[7,8,9] It seemed of interest to confirm these results on Drosophila, and a number of experiments have been carried out by two different methods: bathing of eggs, and injection into adult flies.

TABLE 1

THE FREQUENCY OF SEX-LINKED LETHALS IN THE GERM CELLS OF ♂♂ WHICH HAD BEEN IMMERSED IN FORMALIN SOLUTION DURING THE EGG STAGE

Chorion	Concentr. of CH_2O in %	Time of exposure in hours	Appr. age of embryos in hours	No. of tested ♂♂	No. of tested spermatozoa	No. of lethals
present	5	2	17	35	372	1
"	4	1	5	33	340	0
absent	4	1	20	18	276	3
"	4	1	5	11	232	1
"	2	1	6	12	261	0

(1) *Bathing of Eggs.* This method has already been used by Rapoport, who, after one-hour treatment, obtained 2–3% sex-linked lethals with a 2% solution, and 5–6% with a 4% one. As table 1 shows, this result could not be confirmed in the present tests.

There is only one series—the first in the second part of the table—which suggests a possible effect of the treatment. Three out of 18 treated ♂♂ had one lethal each, resulting in an overall mutation rate of 1.1%, a value which is slightly in excess of the usual mutation rate of the stock used (about 0.3%). The increase is, however, statistically not significant and considerably smaller than that reported by Rapoport for the same kind of

treatment. Moreover, treatment in this series was given at a stage when the fully formed larvae is present in the egg, ready to emerge: it seems possible that small amounts of formaldehyde which have penetrated through the vitelline membrane may be swallowed by the emerging larvae and, as in the feeding tests, reach the germ cells *via* the digestive tract.

This certainly must have been so in the one series, not listed in table 1, in which a considerable effect was obtained: 23 lethals occurred in 309 chromosomes, gives a mutation rate of 7.4%. In this series, an agar slab with eggs which had been deposited on it 16–20 hours earlier was flooded for 2 hours with a 5% solution of formaldehyde. Subsequently, the slab was washed by repeated flooding with tap water, and finally the eggs were scraped off the surface and transferred to food vials. Since no mutations were obtained from a sample of the same treated eggs which had been cleaned and washed individually before transfer to food, it is clear that the mutagenic effect must have been caused by formaldehyde which had been carried into the food with the tiny scraps of agar transferred on the eggs. Thus in the only clear positive series treatment had been given to young larvae by mouth and not to embryos through diffusion. If Rapoport should have used a similar technique in his egg bathing experiments, the discrepancies between his experiments and those described here might find a simple explanation.

(2) *Injection into adult flies.* 1–3 days old ♂♂ received abdominal injections of Drosophila Ringer solution containing from 0.03 to 0.5% formaldehyde. No mutagenic effect was obtained with 0.03, 0.06, and 0.1%. With 0.2% there was a slight, but not significant increase in mutation rate. Clear positive results were obtained in two tests on ♂♂ with 0.3%: In the first, 319 X-chromosomes from 16 treated ♂♂ contained 9 lethals (2.8%); in addition there was one gonadic mosaic for a lethal and one sex-linked visible mutation. In the second experiment, 460 chromosomes from 11 treated ♂♂ contained 19 lethals (4.1%) and 2 semi-lethals. Treatment with 0.4% yielded only 3 fertile ♂♂, which between them produced 2 lethals in 79 chromosomes, a rate of 2.5%. Treatment with 0.5% proved too toxic for use. No mutations were produced by injection of a 0.3% solution into young ♀♀ with still undeveloped ovaries: only 1 sex-linked lethal occurred in 796 chromosomes from 8 such ♀♀, 4 of which had lived long enough to produce progeny for two weeks following injection. Curiously enough, there was no lethal in 164 spermatozoa which had been treated by injection of 0.3% formaldehyde into inseminated ♀♀.

It thus is clear that free formaldehyde can induce mutations in Drosophila germ cells. There are, however, differences between its effect and that of formaldehyde food. The first is the relative inefficiency of free formaldehyde, which in 0.3% solution induced between 3 and 4% sex-linked lethals, while a 0.2% admixture of formaldehyde to the food produces on the average 6–7% lethals, and rarely less than 4%. More important is the second difference, which refers to the stage at which the germ cells respond to the treatment. Whereas the feeding method acts preferentially on young stages,

which already in old larvae have been passed by the most advanced germ cells in the testis,[6] injection affects mature sperm and has only a very slight effect on younger stages. This was shown by the fact that the great majority of mutations in the injection experiments occurred in the first brood, which had been obtained by keeping each treated male with 3 ♀♀ for 3 days after injection. Later broods, produced by a succession of new ♀♀, had small or negligible proportions of lethals. The data for the two experiments with 0.3% are shown in table 2.

TABLE 2

THE FREQUENCY OF SEX-LINKED LETHALS IN SUCCESSIVE BROODS (3 ♀♀ EVERY 3 DAYS) FROM ♂♂ WHICH HAD BEEN INJECTED WITH 0.3% FORMALDEHYDE

Expt.	Days after treatment									
	0–3		3–6		6–9		9–12		12–15	
	n	Leth. %	n	Leth. %	n	Leth. %	n	Leth. %	n	Leth. %
1	319	2.8	345	0.6	147	0.7	309	0.3	188	1.1
11	460	4.1	307	1.3	88	0				

The possible significance of this difference between the effects of formalin feeding and formalin injection has been more fully discussed elsewhere,[10] and the possibility has been considered that these two techniques differ not so much quantitatively in strength and duration of treatment, as qualitatively in the mechanism by which they induce genetical effects.

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July 14, 1952

PUBLICATIONS RECEIVED

THE AMERICAN NATURALIST is glad to acknowledge here the receipt of books on biological and natural history subjects which are likely to be of interest to our readers. No undertaking to publish reviews is implied in this acknowledgment. Books for notice may be sent to:

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Albritton, Errett C., Editor, 1951. Standard values in blood. A. F. Technical Report No. 6039, U. S. Air Force, Wright-Patterson Air Force Base, Dayton, Ohio.

Bonner, J. T., 1952. Morphogenesis: an essay on development. 296 p., 89 figs. $5.00. Princeton University Press, Princeton, New Jersey.

Carr, Archie, 1952. Handbook of turtles. 542 p., 37 figs., 82 plates. $7.50. Comstock Publishing Associates, Ithaca, New York.

This latest of the Comstock Series essentially replaces the only previous book on North American turtles, that by Clifford Pope (1939). It goes, indeed, a great deal beyond that book which was concerned primarily with turtul habits. In it we have at last a long needed summary of what is at present known about the systematics, distribution, ecology, and economic value of this relatively neglected group. This considerable mass of fact is presented in a simple, attractive, often casual and occasionally facetious style by a man who has done important systematic studies on several of the genera and whose personal acquaintance with the animals is evident in every part of the book.

Photographs and diagrams are very numerous and for the most part very useful. Maps of geographic range, as a matter of the author's policy, report ranges as continuous even when the evidence is not adequate. A number of subspecies are included about which the author is properly skeptical, but which he prefers to discuss rather than suppress. There is an attempt to include information on the fossil record, but the result is neither very complete nor very critical. A revised nomenclature for the horny scutes and bony plates of the turtle shell, credited to the late Dr. Leonhard Stejneger, is adopted; it may be doubted whether this will achieve general use. While the discussion of species has been carefully brought up to date, the subordinal distinction granted the leatherback turtle seems oldfashioned.

It is interesting to check the number of genera in which, even in this semi-popular book, the author admits to taxonomic problems. These are

seven of the 14 non-marine genera, a neat half. If one goes further and notes the frequent admissions of our ignorance of habits and other non-taxonomic matters, it becomes evident that there is much still to do before this section of our North American fauna becomes really well known.

These frank statements of our ignorance are a real virtue of the book, and the book in many other ways fills a definite need. It will now for the first time be possible for the inexperienced to identify North American turtles with reasonable, if not perfect, accuracy, and it will no longer be necessary for the physiologists *after* they have performed their experiments to come to the specialist, inquiring on what species they have been working and whether they have one or two species.

E. W.

Collins, Henry H., Jr., 1952. The unvanquished buffalo. 16 p., 8 ill. 25¢. Blue Heron Press, Bronxville, New York.

This pamphlet, which gives an account of the life and fate of the American bison, is designed for distribution by wildlife and natural history groups. It seems accurately and interestingly written.

M. B.

Dunn, L. C., and Th. Dobzhansky, 1952. Heredity, race and society, revised ed. 143 p. 35¢. New American Library (Mentor Books), New York.

Engle, Earl T., editor, 1952. Studies on testis and ovary, eggs and sperm. 237 p., ill. $7.75. Charles C. Thomas, Springfield, Illinois.

This volume contains 14 papers presented at a conference in May, 1950, sponsored by the Committee on Human Reproduction, National Research Council. Seven papers deal with sperm and spermatogenesis in man, five with ovum and ovary in humans and experimental mammals, and two with hereditary semi-sterility and quasi-sterility in the mouse. Each paper is followed by a transcript of the discussions by members of the conference who included 98 specialists from the U. S. and other countries.

L. C. D.

Harkins, William D., 1952. Physical chemistry of surface films. 413 p., ill. $10.00. Reinhold Publishing Corporation, New York.

Hull, Helen S., 1952. Wild flowers for your garden. 280 p., ill. $4.95. M. Barrows and Company, Inc., New York.

A clearly written, well-illustrated book about those American wild flowers which can be grown in home gardens, with lists of species for each of the 48 States (it is surprising how many can be grown throughout this great area), a list of the twelve most popular, directions for cultivation and propagation, bibliography and index; in general a palatable combination of information and inspiration.

L. C. D.

Linssen, E. F. editor, 1951. Nature interlude. A book of natural history quotations. 256 p. 12/6. Williams and Norgate, Limited, London, W. C. 1.

A fascinating assemblage of short quotations about nature drawn from a truly vast variety of literary and scientific sources. For example, the section "Biological Control" begins with a "Pensee" of Pascal: "Nature has placed us so well in the middle that if we change one side of the balance we also change the other." It includes quotations from Pope, Coleridge, Emerson, Vauvenargues, Georges Duhamel, Tennyson, Bacon, Swift, Calverley, Wordsworth, and the Cactus and Succulent Journal of America, which yields the following question epitomizing all those unanswerable ones which make the balance of nature such an unstable one:

When the pear pest in the past is,
Who will blast the Cactoblastis?"

L. C. D.

Lorenz, Konrad Z., 1952. King Solomon's ring. 202 pp., ill. $3.50. Thomas Y. Crowell Co., New York.

A charming book—the text easily translated from the German into an absorbing account of Dr. Lorenz' adventures with his animals, with delightful Lorenzian drawings on every page. No biologist will be bored with the book, which gives considerable insight into Lorenz' theories of behavior without recourse to any technicalities. Among laymen, the book ought to interest anyone who likes birds, or has an aquarium, or even who takes an intelligent interest in his dog. The last chapter has some thoughtful comments on the aberrant behavior of the human animal.

M. B.

Morton, Dudley J. (with Dudley Dean Fuller), 1952. Human locomotion and body form. 285 p., ill. $5.00. Williams and Wilkins Co., Baltimore, Maryland.

A discussion of the biomechanics of the human foot and of the process of walking resulting from the collaboration of an anatomist and a mechanical engineer.

L. C. D.

Smith, A. C., 1952. Studies of Pacific Island plants, X. The Meliaceae of Fiji, Samoa and Tonga. Contributions from the U. S. National Herbarium, Vol. 30, Part 4, pp. 469-522. 50¢. Smithsonian Institution, Washington, D. C.

Srb, Adrian M. and Ray D. Owen, 1952. General genetics. 561 p., ill. $5.50 W. H. Freeman and Company, San Francisco.

A text book which is well-written and illustrated, up-to-date and apparently complete enough for a one year course at the upper college level. Although it deals primarily with principles there are chapters on plant and

animal breeding and on human genetics. The subjects covered are extended by the many problems accompanying each chapter and by good bibliographies and indexes.

L. C. D.

Strausbaugh, Perry D., and Bernal R. Weimer, 1952. General biology, 3rd edition. 813 p., ill. $6.00. John Wiley & Sons, Inc., New York.

Zahl, Paul A., 1952. Flamingo hunt. 270 p. $3.50. Bobbs-Merrill Co., Indianapolis.

A narrative of three trips to the Bahamas in search of flamingo rookeries. There are vivid descriptions of the salt marshes and lakes of Andros and Inaugua Islands, with glimpses of the people who manage to subsist there and of the marine fauna, which flourishes, and considerable interesting detail on the ecology of flamingos and on the possible causes of their decline in numbers.

M. B.